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Experimental Simulation of an Estrous Cycle — Gonadotropin Surges in Estradiol-Infused Ovariectomized Rats

by

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ABSTRACT

Dynamic relations between the circulating estrogen and the hypophyseal gonadotropin secretion in the estrous cycle were investigated by replacing the ovaries by an infusion pump in freely moving rats. Female rats were ovariectomized in the morning at certain stages of the 4-day estrous cycle, and simultaneously infused with estradiol (E_2) at a constant rate of 0.35 ng/min up to 120 h through a cannula chronically inserted into the jugular vein. They were killed at 6 h-intervals. Rats ovariectomized at the second day of diestrus and at estrus showed a sharp rise in LH 36 h and 84 h, respectively, after the initiation of E_2 infusion, when the proestrous surge would occur in normal rats. During the other periods, blood levels of LH were very low, exhibiting a small daily rise in the evening. Similarly ovariectomized rats infused with vehicle only showed a gradual rise of gonadotropin secretion, never reaching the surge level. Rats ovariectomized at proestrus and infused with E_2 showed a LH surge 12 h later as expected. However, surge-like LH secretions followed every evening thereafter. Thus, the constant supply of E_2 alone could simulate at least one 4-day cyclic LH surge in ovariectomized rats. E_2 infusion caused a daily peak of FSH synchronized with the LH rises, but could not suppress the post-operative hypersecretion. It is discussed that if the suppressing effect of progesterone endogenously secreted from the ovaries is cleared, a circadian pattern of the LH/FSH surge may appear under the signal from the cerebral clock mechanism and the effect of circulating estrogen. The failure to suppress the FSH hypersecretion by E_2 might indicate the involvement of inhibin in the regulatory mechanism. Time-course changes in uterine and vaginal weights are also dealt with and discussed in relation to the constant E_2 exposure.

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INTRODUCTION

The estrous cycle is controlled by the interplay of many components in the regulatory system (Brinkley, 1981). The most important components are the brain, hypophysis and ovary. The brain processes a number of neural and humoral inputs from the external and internal environments, and sends control signals in the form of neurohormones to the hypophysis. The hypophysis secretes gonadotropins and prolactin which induce the functional and structural changes of the ovary. The ovary in turn modifies the activity of the brain and the hypophysis through its hormone secretions. The ovarian changes proceed sequentially to exert a clock function in the estrous cycle. The environmental daily light-dark cycles entrain the biological clock mechanism in the brain, which adjusts the timing of the ovulation-inducing surge of gonadotropin secretion. Such a feedback relation among these components makes the time-course analysis of the regulating system difficult, since the state of the system changes dynamically.

It might be useful to dissect the feedback loop in the regulatory system of the estrous cycle by removing the interdependency. The replacement of an endocrine organ by an infusion pump may allow the artificial control of blood hormone levels, the program of which is never altered by the state changes in the biological system. The senior author developed a technique to control the blood level of hormones in unrestrained rats (Inoué, 1970) and has applied it to the analyses of dynamic reproductive events (reviewed by Inoué, 1981a). The present paper deals with a similar approach to the dynamic relations between the ovary and the brain-hypophyseal system and between the ovary and the reproductive organs. Preliminary studies were published elsewhere in an abstract form (Inoué and Wada, 1980).

MATERIALS AND METHODS

Four hundred and forty-five female rats of the Sprague-Dawley strain were used. They were 70-90 days of age, weighing 200-250 g, being maintained under LD 12:12 (L: 0800-2000) in an air-conditioned room at 25 ± 1°C, 50 ± 10% relative humidity. Vaginal smears were daily monitored and the surgical operation was performed at 0830-1000 at a certain stage of 4-day estrous cycles, i.e. proestrus (P), estrus (E) and the second day of diestrus (D_2). Rats were ovariectomized under ether anesthesia and simultaneously implanted with an intrajugular cannula, a thin polyethylene tubing (PE 50, Intramedic) protected at the tip by a Silastic tubing (602-155, Dow Corning) according to a slightly modified method of Inoué (1970).

Soon after the operation, which was finished within 15 min, rats were

individually housed in a special cage (20 x 30 x 35 cm) and estradiol-17β (E_2) dissolved in propylene glycol were continously infused through the cannula. Pumping rate was fixed as 0.35 ng E_2/1.3 μl/min (= 21 ng E_2/75 μl/h = 0.50 μg E_2/1.8 ml/day). Similarly ovariectomized rats infused with vehicle alone were also prepared. A cannular feedthrough swivel (Muromachi-Kikai) inserted between the infusion pumps (Unita II, Braun) and the cannula guaranteed the free movement of animals. At either 6 h or 12 h intervals (0830-1000, 1430-1600, 2030-2200 and 0230-0400), 5 rats in each group were decapitated to collect the trunk blood, which was allowed to clot at room temperature for 1 h and overnighted in a refrigerator. After centrifugation at 3000 r.p.m. for 15 min, serum preparations were stored in a deep freezer at —20°C until the radioimmunoassay (RIA) of gonadotropins. Weights of the uterus and vagina were measured by an analytical balance (2492, Sartorius) up to 0.1 mg and expressed as 100 g body weight. Dynamic changes in the weights of the hypophysis and adrenals are analyzed and reported elsewhere (Inoué, 1981b). Time-course changes in serum prolactin will be dealt with elsewhere (Inoué and Wada, in preparation).

Serum concentrations of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were determined by the standard procedures using the RIA kits provided from the Rat Pituitary Hormone Distribution Program, National Institute of Arthritis, Metabolism and Digestive Diseases (NIAMDD) and Dr. A. F. Parlow. The reagents used were NIAMDD-Rat-FSH-I-3 and NIAMDD-Rat-LH-I-4 or I-7 for radioiodination, antibodies A-Rat-FSH-S-8 and A-Rat-LH-S-4 for the first antibodies in the appropriate double-antibody RIA, and Rat-FSH-RP-1 and Rat-LH-RP-1 for reference preparations. The results were expressed in terms of μg equivalents/ml NIAMDD-Rat-FSH-S-1 and ng equivalents/ml of NIAMDD-Rat-LH-S-1.

Statistical analyses of results were done by microcomputer systems (Sharp and Apple II).

RESULTS

LH secretion in E_2- and/or vehicle-infused ovariectomized rats

Rats ovariectomized in the morning of D_2 and infused with vehicle alone exhibited a gradual rise in serum LH levels, accompanying some time-to-time fluctuations. In contrast, similarly treated females infused with E_2 maintained low LH levels for the initial 30 h as seen in normal females. At 36 h after the initiation of the treatment, i.e. at 2030-2200 of the second night, a sharp surge-like LH rise occurred. This timing exactly accorded with that of the LH surge in normal cyclic females. After then, LH levels returned to low values also as in normal rats except for small but significant rises at 2030-2200 (Fig. 1).

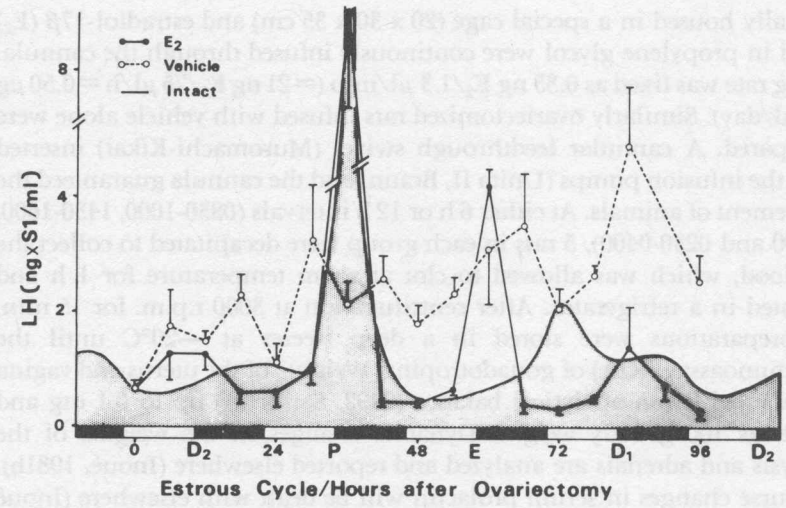

Fig. 1. Time-course changes in serum LH in rats ovariectomized at D_2 and infused with E_2 and/or vehicle alone. Black and white bars beneath the abscissa stand for the environmental light and dark periods, respectively. Each point and attached vertical line represent respectively mean and standard error of 5 rats. Shadowed area indicating the averaged level in normal 4-day cyclic rats is constructed from data accumulated in our laboratory.

If rats were ovariectomized at E and infused with vehicle alone, serum concentrations of LH remained low for the initial 48 h and then increased gradually with some ups and downs. E_2 infusion caused a pattern of LH secretion almost similar to vehicle-infused rats for the first 60 h, except for a small but distinct peak at 2030-2200 appearing 36 h and 60 h after the initiation of the infusion. A sharp surge-like LH rise occurred at 2030-2200 of the 4th night, which exactly corresponded with the time of the LH surge in normal cyclic rats (Fig. 2).

Vehicle-infused rats ovariectomized at P clearly demonstrated a rapid rise in LH concentrations in 6 h prior to a LH surge which took place 12 h after the operation. Thereafter, levels of serum LH fell to those in normal rats and then rose slowly. E_2-infused females demonstrated a sharp surge-like LH rise 12 h after the operation, which exactly accorded with the time of the surge in normal cyclic rats. In addition, from the next day on, their blood LH levels showed falls in the morning and surge-like rises in the evening (Fig. 3).

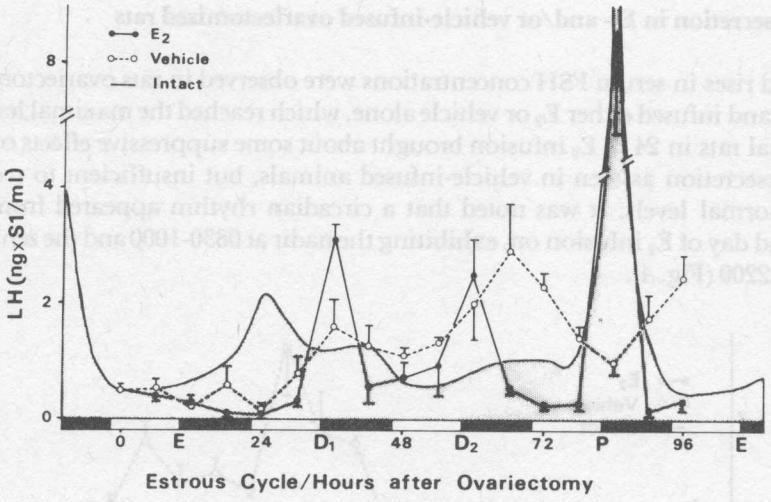

Fig. 2. Time-course changes in serum LH in rats ovariectomized at E and infused with E_2 and/or vehicle alone. Other explanations, see Fig. 1.

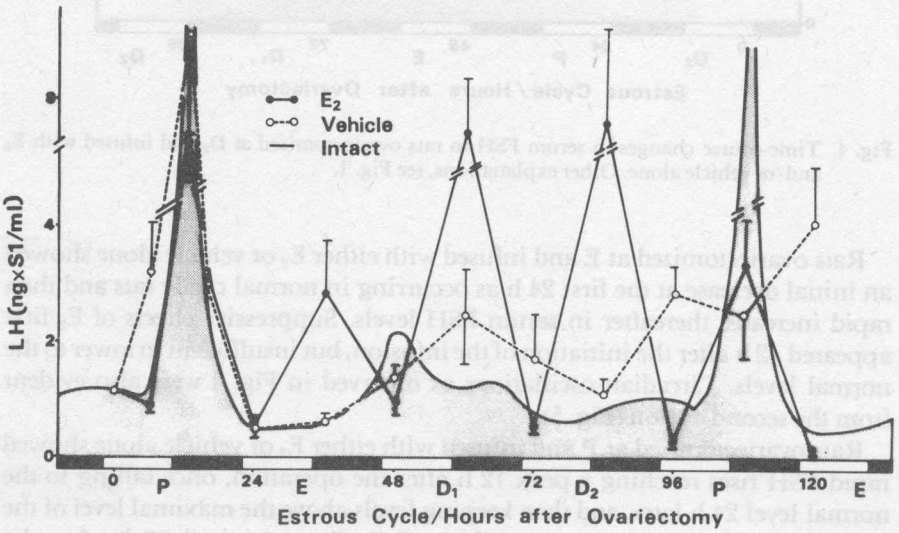

Fig. 3. Time-course changes in serum LH in rats ovariectomized at P and infused with E_2 and/or vehicle alone. Other explanations, see Fig. 1.

FSH secretion in E_2- and/or vehicle-infused ovariectomized rats

Rapid rises in serum FSH concentrations were observed in rats ovariectomized at D_2 and infused either E_2 or vehicle alone, which reached the maximal level in normal rats in 24 h. E_2 infusion brought about some suppressive effects on the hypersecretion as seen in vehicle-infused animals, but insufficient to recover the normal levels. It was noted that a circadian rhythm appeared from the second day of E_2 infusion on, exhibiting the nadir at 0830-1000 and the zenith at 2030-2200 (Fig. 4).

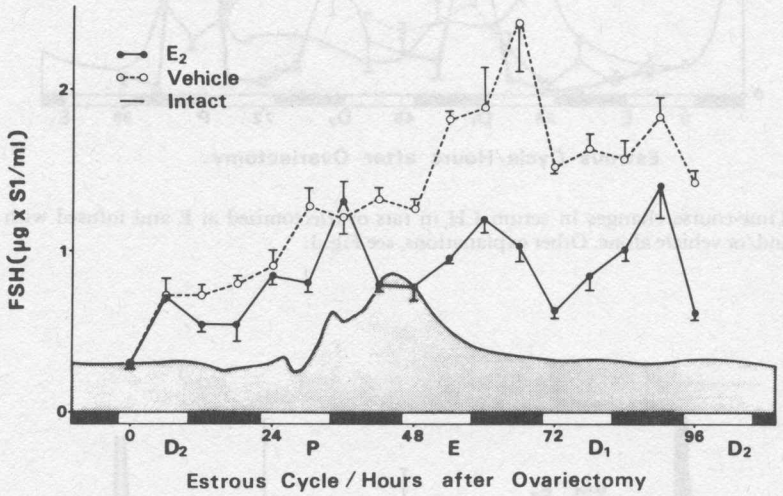

Fig. 4. Time-course changes in serum FSH in rats overiactomized at D_2 and infused with E_2 and/or vehicle alone. Other explanations, see Fig. 1.

Rats ovariectomized at E and infused with either E_2 or vehicle alone showed an initial decrease at the first 24 h as occurring in normal cyclic rats and then rapid increases thereafter in serum FSH levels. Suppressive effects of E_2 first appeared 42 h after the initiation of the infusion, but insufficient to lower to the normal levels. Circadian oscillations as observed in Fig. 4 were also evident from the second day on (Fig. 5).

Rats ovariectomized at P and infused with either E_2 or vehicle alone showed rapid FSH rises reaching a peak 12 h after the operation, once falling to the normal level 24 h later, and then keeping levels above the maximal level of the normal cyclic rats. Suppressive effects of E_2 first appeared 48 h after the initiation of the infusion, accompanying the apparant circadian rhythmicity from the third day on (Fig. 6).

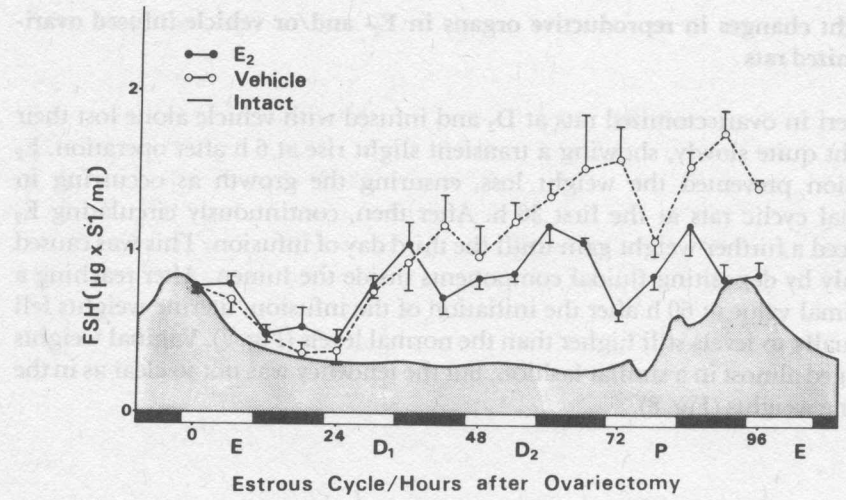

Fig. 5. Time-course changes in serum FSH in rats ovariectomized at E and infused with E_2 and/or vehicle alone. Other explanations, see Fig. 1.

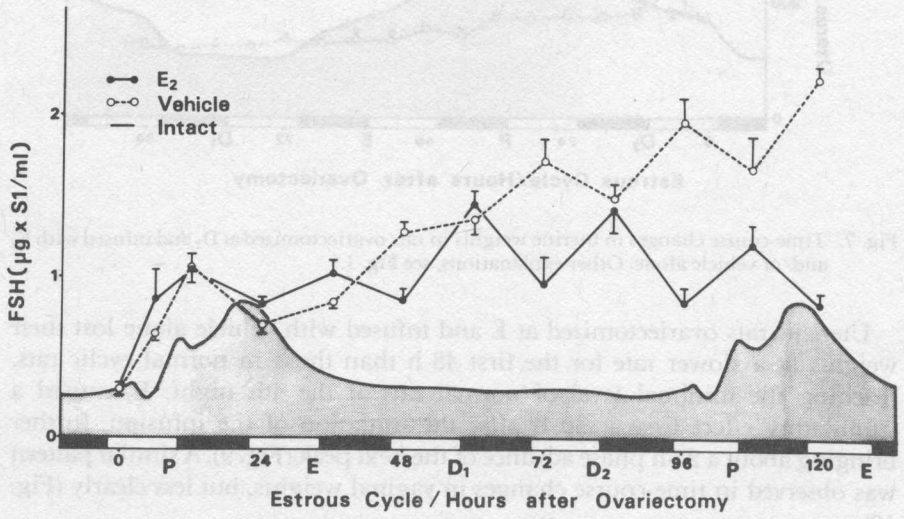

Fig. 6. Time-course changes in serum FSH in rats ovariectomized at P and infused with E_2 and/or vehicle alone. Other explanations, see Fig. 1.

Weight changes in reproductive organs in E_2- and/or vehicle-infused ovariectomized rats

Uteri in ovariectomized rats at D_2 and infused with vehicle alone lost their weight quite slowly, showing a transient slight rise at 6 h after operation. E_2 infusion prevented the weight loss, ensuring the growth as occurring in normal cyclic rats at the first 36 h. After then, continuously circulating E_2 induced a further weight gain until the third day of infusion. This was caused mainly by depositing fluidal components inside the lumen. After reaching a maximal value at 60 h after the initiation of the infusion, uterine weights fell gradually to levels still higher than the normal levels (Fig. 7). Vaginal weights changed almost in a similar fashion, but the tendency was not so clear as in the uterine weights (Fig. 8).

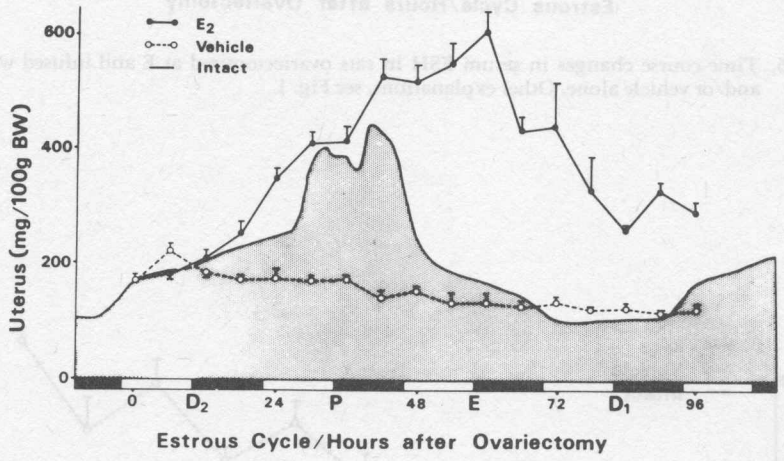

Fig. 7. Time-course changes in uterine weights in rats ovariectomized at D_2 and infused with E_2 and/or vehicle alone. Other explanations, see Fig. 1.

Uteri in rats ovariectomized at E and infused with vehicle alone lost their weights at a slower rate for the first 48 h than those in normal cyclic rats, reaching the minimal level of normal rats at the 4th night. E_2 caused a stimulatory effect first at 30 h after the initiation of the infusion, further bringing about a 24-h phase advance of the next peak (Fig. 9). A similar pattern was observed in time-course changes in vaginal weights, but less clearly (Fig. 10).

Uteri in rats ovariectomized at P and infused with vehicle alone showed a weight increase similar to normal cyclic rats at the first 24 h and then a gradual

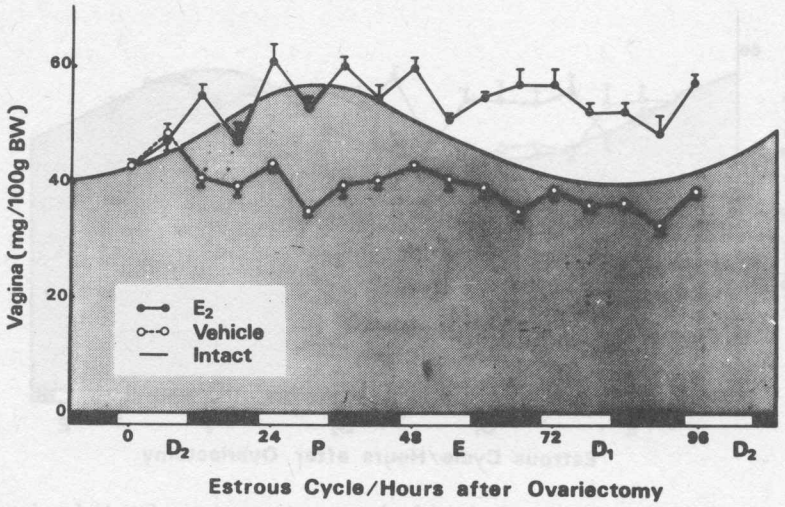

Fig. 8. Time-course changes in vaginal weights in rats ovariectomized at D_2 and infused with E_2 and/or vehicle alone. Other explanations, see Fig. 1.

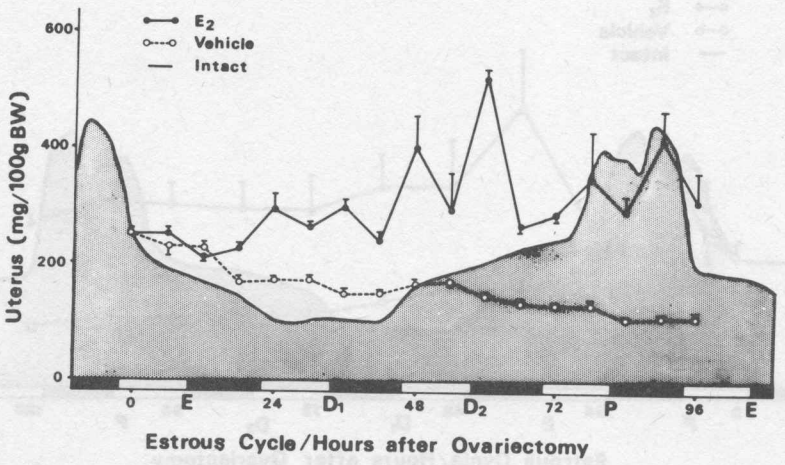

Fig. 9. Time-course changes in uterine weights in rats ovariectomized at E and infused with E_2 and/or vehicle alone. Other explanations, see Fig. 1.

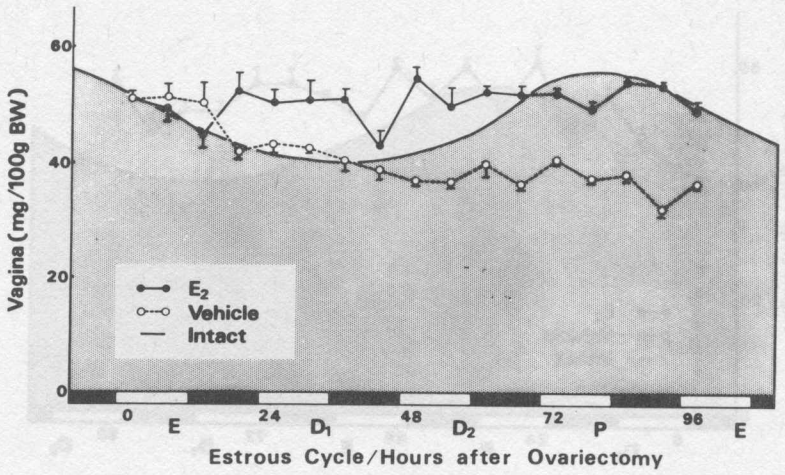

Fig. 10. Time-course changes in vaginal weights in rats ovariectomized at E and infused with E_2 and/or vehicle alone. Other explanations, see Fig. 1.

decrease afterward. On the second day of infusion, E_2 brought about another and higher peak, and then maintained a steady weight which was near to the maximal weight in the normal cyclic rats (Fig. 11). A similar but less apparent tendency was observable in vaginal weight changes (Fig. 12).

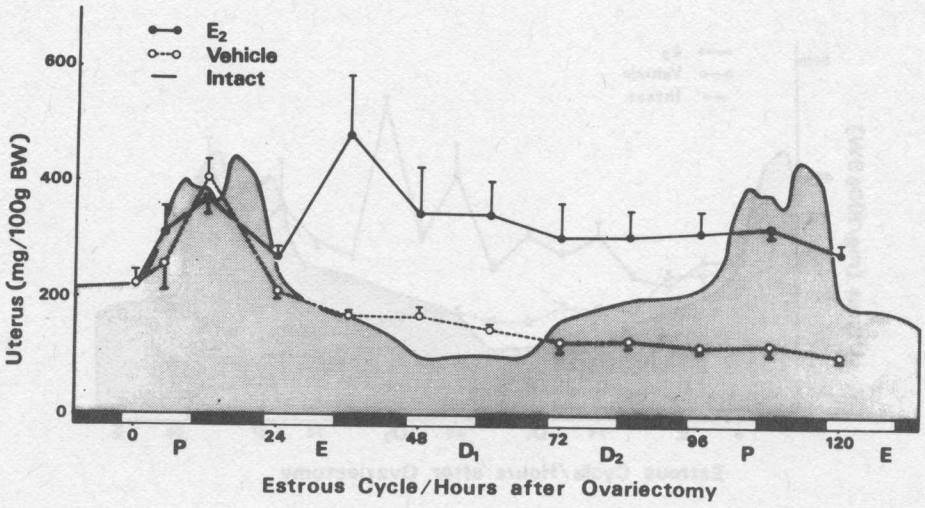

Fig. 11. Time-course changes in uterine weights in rats ovariectomized at P and infused with E_2 and/or vehicle alone. Other explanations, see Fig. 1.

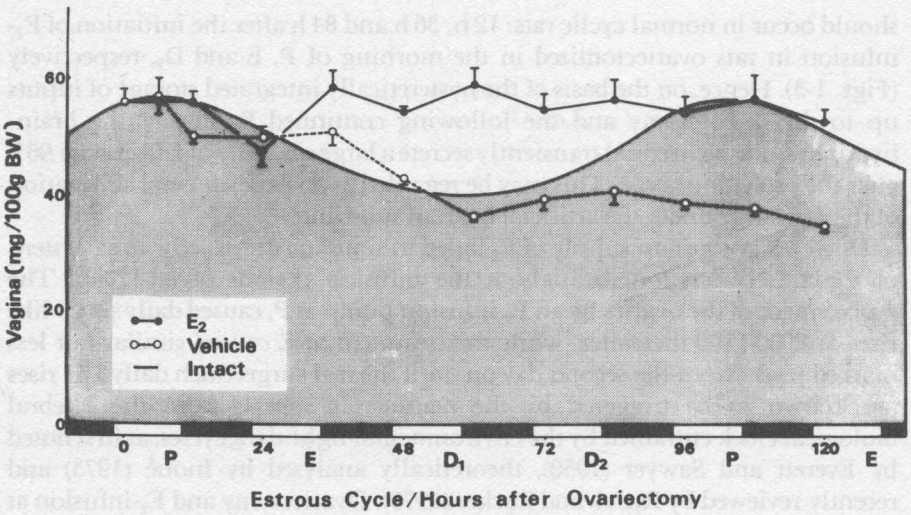

Fig. 12. Time-course changes in vaginal weights in rats ovariectomized at P and infused with E_2 and/or vehicle alone. Other explanations, see Fig. 1.

DISCUSSION

The rat estrous cycle is characterized by cyclic secretions of hypophyseal and ovarian hormones (Butcher et al., 1974). In the present study, in which the ovaries cyclically secreting estrogen and progestogen were replaced by an infusion pump acyclically supplying E_2 only or vehicle alone, the brain-hypophyseal system and the reproductive organs reacted in a different way according to the different stage of the estrous cycle.

Different secretory patterns of LH as an early response to ovariectomy at different stages of the estrous cycle are reported by Yamamoto et al. (1970) and Tapper et al. (1972). However, they do not indicate the time of operation and their sampling intervals are quite long (once daily in most cases). The present study further demonstrated more distinct differences in time-course changes in not only LH but also FSH and weights of the uterus and vagina. In addition, it was revealed that also under the constant E_2 supply these parameters showed a striking dependency on the initial state, i.e. the time of the operation.

E_2-LH dynamics

Changes in serum LH were most dramatic. The "artificial ovary" supplied E_2 alone at a physiological rate, providing neither 4-day cyclicity nor progesterone. Nevertheless, at least one LH surge was induced at the very time when it

should occur in normal cyclic rats: 12 h, 36 h and 84 h after the initiation of E_2-infusion in rats ovariectomized in the morning of P, E and D_2, respectively (Figs. 1-3). Hence, on the basis of the hysteretically integrated storage of inputs up to the ovariectomy and the following continued E_2 inputs, the brain-hypophyseal system could transiently secrete a large quantity of LH exactly 96 h after the preceding surge. This may be regarded as an experimental simulation of the LH surge under the artificial ovarian substitute.

However, a constant supply of E_2 failed to simulate the exactly same pattern of tonic LH secretion throughout the infusion periods of 96-120 h. The replacement of the ovaries by an E_2 infusion pump at P, caused daily surge-like rises at 2030-2200 thereafter, while the treatment at E caused similar but less marked peaks from the second day on until the real surge. Such daily LH rises are known to be triggered by the neurogenic signals from the cerebral biological clock entrained by the environmental light-dark cycles, as first noted by Everett and Sawyer (1950), theoretically analyzed by Inoué (1973) and recently reviewed by Rusak and Zucker (1979). Ovariectomy and E_2-infusion at D_2 caused similar rises occurring daily after the surge. Similar results are reported to demonstrate the existence of a daily signal for the LH surge in estrogen-implanted ovariectomized rats (Legan and Karsch, 1975; Banks and Freeman, 1980) and the evidence of a critical period for the signal in the afternoon of diestrus as revealed in estrogen-primed ovariectomized rats (Geiger et al., 1981). It may be speculated that the lack of 4-day cyclicity in the infusion program and no simultaneous supply of progesterone may be responsible for the occurrence of the unusual LH "mini-surges".

A possibility that the duration of the suppressive effect of progesterone in the preceding cycle may determine the period of the following cycle, is theoretically suggested by Inoué (1973) and experimentally demonstrated in goats by Gidley-Baird (1980). The quantity and duration of effects of progesterone on the brain-hypophyseal system differ according to the stage of the estrous cycle. Since little progesterone had been secreted before the treatment at P, the brain-hypophyseal system escaped from the suppressive effects of progesterone to allow daily surge-like rises under the priming effect of constantly circulating E_2. The stage E is characterized by the maximal secretion of progesterone (Butcher et al., 1974), which serves to abolish the estrogen-induced daily signal for the LH surge (Banks and Freeman, 1978). In the present experiments, the progesterone secretion exerted relatively long-lasting suppressive effects on the gonadotropin secretion in both vehicle- and E_2-infused ovariectomized rats. Although small peaks appeared from the second day on under E_2 exposure, 3 days were required to initiate the next real surge. Similarly in rats ovariectomized at D_2, another peak of progesterone coming at D_2 (Butcher et al., 1974) might be responsible for low LH levels at the first night of ovariectomy. Decrements of daily LH mini-surges following the real surge

might be accounted for by the lack of changes in E_2 levels as suggested by Legan et al. (1975).

Soon after the ovarian outputs were nullified in the morning of P, serum LH levels increased precedently to the normal surge (Fig. 3). Tapper et al. (1972) first noted the phenomenon and attributed it to the effect of progesterone secreted by the adrenals as a result of the surgical stress. However, similarly operated and E_2-infused rats did not show such a LH rise until the time of normal surge. Hence, it is likely that this was caused by the withdrawal of estrogen. This speculation may be supported by Turgon (1979). She demonstrated that when the decline in E_2 is prevented by implantation of E_2-containing capsule during the rising phase of the LH surge, the magnitude of the surge is decreased.

E_2-FSH dynamics

Time-course changes in serum FSH in ovariectomized and E_2 and/or vehicle infused rats showed also a marked dependency on the time of the operation during the estrous cycle. A post-operative increase was rapidly induced in rats treated in the morning of P and D_2, while it appeared first 30 h after the operation in rats treated at E. Regardless the time of the operation, serum levels became higher than the maximal level in normal cyclic rats within 36 h after the treatment. These high levels of serum FSH in vehicle-infused ovariectomized rats showed a great contrast to those of serum LH, which never reached the surge level in 96-120 h.

Constant supply of E_2 exerted less suppressive effects on serum FSH levels than on serum LH levels. Higher levels than those in normal cyclic rats were maintained throughout the infusion periods. However, similarly as in the LH secretion, a circadian pattern in FSH secretion was clearly induced by E_2 infusion. The timing of the occurrence of peaks exactly coincided with that of real and mini LH surges, suggesting that the same biological clock mechanism, govering the LH release, was functional also daily in the FSH surges. Here also the integrated information up to the treatment, including the hysteretic suppressive effect of progesterone, was reponsible for the differences in the time-course secretory patterns in E_2-infused rats ovariectomized at different stages of the estrous cycle.

The failure to recover the normal basal levels of serum FSH by E_2 may support the theory of Shander et al. (1980) that, in addition to the ovarian steroids, inhibin is involved in the regulation of FSH secretion. Since the "artificial ovary" supplied only E_2 and/or vehicle, no inhibin was available in the present experiments. The high levels of serum FSH might be attributed in part to the absence of inhibin. Butcher (1977) suggests that the decrease of ovarian inhibin is responsible for the surge of FSH. A rapid post-operative rise of FSH at P (Fig. 6) might be accounted for by such a mechanism.

E_2-reproductive organ dynamics

The dependency on the initial state at the time of the operation was also striking in the weight changes in the reproductive organs, the targets of ovarian sex hormones. Uteri in rats ovariectomized at P had already acquired a potency to grow in a normal fashion in the first 24 h regardless the absence or presence of E_2. The first weight peak as in normal cyclic rats was followed by a second and higher peak in subsequent 24 h. Thereafter, uteri maintained a steady weight. Similar oscillatory response of uterine weights responding to constant E_2 inputs in 48-h ovariectomized rats are previously reported (Inoué and Sekiguchi, 1970) and mathematically analyzed (Inoué, 1973).

Ovariectomy and the initiation of E_2 infusion at E resulted the maintenance of the initial weight for 2 days and a 24-h phase advance in reaching the peak. This may indicate that the uterus had a temporal capacity to complete the maximal growth in 72 h after the preceding peak under the estrogenic stimulation. In comparison with normal cyclic rats, vehicle-infused rats showed a delay in losing their uterine weights for the first 2 days (Fig. 9). It is speculated that the weight loss could be accelerated by the presence of ovarian factors, possibly progesterone secreted during the luteal phase.

Ovariectomy and the initiation of E_2 infusion at D_2 caused a normal-typed uterine growth for the first 40 h. The growth still continued until the third day. This fact may indicate that the uterine tissues had a temporal capacity to grow 24 h further, and that they had a structural capacity to become ca. 50% heavier. The weight decrease subsequent to the peak was mainly due to the disappearance of the intraluminal fluid.

Time-course changes in the vaginal weights showed a similar but less remarkable characteristics in comparison with those of the uterine weights. A less sensitive nature of vaginal tissues and no retention of fluidal components inside the lumen may account for the difference.

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