Effects of 17α-Hydroxyprogesterone on Luteinizing Hormone Release in the Rat

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Synopsis

Our previous study, in which we measured serum 17α-hydroxyprogesterone (17-OHP) every 8 hr during the periovulatory phase in women, suggested that 17-OHP may have a physiological role in inducing and/or facilitating LH release. Therefore, this study was designed to elucidate the possible role of 17-OHP in LH release.

Experiment 1: Three weeks after ovariectomy, rats were injected subcutaneously with 20 µg of estradiol benzoate (EB) at 1200 hr (day 0). The administration of 2 mg of progesterone (P) or 17-OHP was performed systemically at 1200 hr on day 3. The injection of P elicited a significant increase of serum LH 6 hr later as compared to the serum levels in the control group (p<0.05), but the injection of 17-OHP failed to alter serum LH significantly.

Experiment 2: Three weeks after ovariectomy, rats were injected with EB at 1200 hr. Crystalline P or 17-OHP was inserted through the outer cannula into the brain at 1200 hr on day 3. All animals bearing 17-OHP implants or P implants in the diagonal band of Broca (DBB) exhibited an increase in serum LH 6 hr after implantation. In regard to implants in the medial preoptic area (MPO), all rats with implants of 17-OHP and 4 of 7 with implants of P exhibited an increase in serum LH, whereas both 17-OHP and P implantations in the septum failed to induce an increase in serum LH.

Experiment 3: All rats were injected subcutaneously with 20 µg of estradiol (E2) rather than with EB. Vaginal cornification was observed in 100% at 1200 hr on day 3 after the injection of EB, but in only 5.5% at 1200 hr on day 3 after the injections of E2. In rats with implants in the DBB or the MPO, no significant increase of serum LH was observed after implantation of 17-OHP or P.

These results suggest that the sites of stimulatory feedback action of 17-OHP on LH release are similar to those of P and that the stimulatory effects of 17-OHP are synergistic with estrogen in the induction of the LH surge.

Results from the present study, in conjunction with our previous observation, strongly suggest a physiological role for 17-OHP in inducing the midcycle LH surge in women.

Serum levels of 17α-hydroxyprogesterone (17-OHP) fluctuate during the menstrual cycle in women (Ross et al., 1970; Abraham et al., 1972). The midcycle ovulatory peak and the marked postovulatory increase in serum 17-OHP are a consistent finding, but one of the major problems to be solved is the role of the 17-OHP rise during the periovulatory phase of the menstrual cycle.

Recently, a physiological role of this hormone during the periovulatory phase was investigated. Serum levels of LH, FSH, estradiol (E2), progesterone (P) and 17-OHP were determined by radioimmu-

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no assay at 8 hr intervals during the periovulatory phase (Uemura et al., 1978). The results suggested that 17-OHP may have a physiological role in inducing and/or facilitating LH release in women.

In the present study, crystalline implants of 17-OHP were stereotaxically localized within specific areas of the brain of the rat in order to further elucidate the possible role of 17-OHP on LH-release.

Materials and Methods

In all studies, adult female rats of Wistar strain weighing 200-280 g were maintained under uniform conditions of 22±1.5°C and a photoperiod of 14 hr (0500 to 1900). Specific treatment was as follows:

Experiment 1

Three weeks after ovariectomy, all the rats were injected subcutaneously with 20 μg of estradiol benzoate (EB) in sesame oil at 1200 hr, and this day was arbitrarily defined as day 0. The second administration of steroids was performed systemically at 1200 hr on day 3. The steroid hormones injected subcutaneously were as follows: P (2 mg in sesame oil, Teikoku Hormone Mfg., Co., Ltd.), 17-OHP (2 mg in sesame oil, Teikoku Hormone Mfg., Co., Ltd.) and 20α-hydroxyprogesterone (20-OHP, 2 mg in sesame oil, Steraloids, Inc.). 17-OHP is very insoluble in oil at room temperature. Therefore, the solution of 17-OHP was stored at 50°C until it was administered.

Experiment 2

Intracerebral implantation of crystalline steroids was performed in ovariectomized rats using a double cannula. One week after ovariectomy, a stainless steel tube (0.65 mm od), used as the outer cannula, was stereotaxically oriented unilaterally such that the tip of the cannula was located in (i) the diagonal band of Broca including the nucleus tractus diagonalis (DBB), (ii) the septum (SEPT), (iii) the medial preoptic area (MPO) or (iv) the arcuate nucleus (ARC). The atlases of Albe-Fessard et al. (1966) and Koenig and Klippel (1963) were used as a reference. The distal end of the cannula was fixed to the skull with dental cement. Two weeks after positioning the outer cannula, all the rats were injected subcutaneously with 20 μg of EB at 1200 hr. Crystalline P, 17-OHP or 20-OHP was tapped into one end of an inner cannula (0.35 mm od), which was then inserted through the outer cannula into the brain at 1200 hr on day 3. In control rats, an empty inner cannula was used.

Experiment 3

Experiment 3 was performed in the same way as experiment 1 and experiment 2, except that all the rats were injected subcutaneously with 20 μg of estradiol (E2) in sesame oil rather than with 20 μg of EB.

Blood samples were obtained by heart puncture under light ether anesthesia at 1200 and 1800 hr on day 0, 3, and 4 as previously reported by Kawakami et al. (1978). Serum LH was measured by the double antibody radioimmunoassay method following instructions provided in the radioimmunoassay kit for LH distributed by NIAMDD. LH concentrations were expressed in terms of NIH-LH-S1. All statistical analyses were based upon Student’s t-test. The sites of the implantations were microscopically identified after the rats were killed.

Results

Experiment 1

In ovariectomized rats 20 μg of EB administered at 1200 h on day 0 lowered the high serum LH concentrations by 1800 h of the same day and the values remained low at 1200 h on day 3. Subcutaneous injection of 2 mg of P at 1200 h on day 3 elicited a significant increase of plasma LH 6 hrs later (8.97 ± 3.56 ng/ml, Mean ± SE, p < 0.05) as compared to the serum LH level in the control group (0.56 ± 0.22 ng/ml), but subcutaneous injection of 2 mg of 17-OHP at 1200 h failed to alter serum LH significantly 6 hrs later (2.06 ± 0.65 ng/ml) (Fig. 1).

Experiment 2

In the 3 rats that received P implants in the DBB, all showed marked increases in serum LH. In 4 of 7 rats bearing P implants in the MPO, marked increases in serum LH were also observed. In the 2 rats implanted in the SEPT, serum gonadotropin titers were unchanged at 6 hrs after implantation (Fig. 2). In animals that received 17-OHP implants, all 6 rats with implants in the DBB and 7 of 8 rats
Fig. 1. The mean concentrations and standard errors of serum LH in ovariectomized estradiol benzoate (EB)-primed rats treated with progesterone (P) or 17α-hydroxyprogesterone (17-OHP). * shows that difference from the control is statistically significant (p<0.05).

Fig. 2. Sites of progesterone implantations in ovariectomized estradiol benzoate-primed rats. A solid circle shows the implanted site exhibited a marked increase of serum LH and a solid triangle shows the implanted site exhibited little increase of serum LH.

Fig. 3. Sites of 17α-hydroxyprogesterone implantations in ovariectomized estradiol benzoate-primed rats.
Fig. 4. Sites of 20α-hydroxyprogesterone implantations in ovariectomized estradiol benzoate-primed rats.

Fig. 5. The mean concentrations and standard errors of serum LH in ovariectomized estradiol benzoate-primed rats bearing progesterone (P) or 17α-hydroxyprogesterone (17-OHP) in the diagonal band of Broca (DBB). * shows that difference from the control is statistically significant (p<0.05).

with implants in the MPO showed marked increases in serum LH. Implants of 17-OHP (n=3) in the SEPT did not alter serum LH titers 6 hrs after implantation (Fig. 3). In the cases of 20-OHP implants, 4 of 6 rats with implants in the MPO showed marked increases in serum LH (Fig. 4).

Fig. 5 shows the changes in serum LH following implantation of steroids in the DBB. Significant increases in mean concentrations of serum LH were observed 6 hrs after implantation of P (4.24±1.18 ng/ml, p<0.05) and 17-OHP (5.75±1.45 ng/ml, p<0.05) as compared with the control group (1.16±0.56 ng/ml). In regard to implants in the MPO, a significant increase in the mean serum concentration of LH was observed 6 hrs after the implantation of 17-OHP (7.81±1.75 ng/ml, p<0.005) as compared with the control group (1.77±0.62 ng/ml). Increases in the mean concentration of LH were also observed after the implantation of P (5.65±2.54 ng/ml) and 20-OHP (4.62±1.44 ng/ml) although these were not significantly different from the control values (Fig. 6). No significant increases in the mean serum LH values were observed after the implantations of these hormones into the SEPT (Fig. 7).

Experiment 3

All the ovariectomized rats were checked for cornification of the vaginal epithelium. Vaginal cornification was observed in 25.0% of rats at 1200 h on day 2 and in 100% of rats at 1200 h on day 3 after the injection of EB. However, vaginal cornification was observed in 73.7% of rats at 1200 h on day 2 and in 5.5% of rats at 1200 h on day 3 after subcutaneous injection of E₂.

Serum LH did not increase significantly 6 hrs after a subcutaneous injection of 17-OHP (3.06±0.67 ng/ml) or P (3.37±0.59 ng/ml) as compared with the control
(2.46 ± 0.55 ng/ml). In rats with implants in the DBB, significant increases in mean concentrations of serum LH were not observed 6 hrs after the implantation of 17-OHP (2.41 ± 0.62 ng/ml) or P (4.56 ± 1.62 ng/ml) as compared with the control (2.75 ± 0.36 ng/ml). In rats with implants in the MPO, no significant increases in the mean concentration of LH were observed 6 hrs after implantation of 17-OHP (2.86 ± 0.25 ng/ml) or P (2.38 ± 0.52), as compared with the control (2.19 ± 0.21 ng/ml).

Discussion

The facilitative action of P on LH release in estrous rats or estrogen primed rats was described by Everett (1940), Caligaris (1968) and Swerdloff (1972). Present results confirm the facilitative action of P in female rats primed with EB. The effects of 17-OHP on LH release in humans and rats have also been studied. Leyendecker and Nocke (1973) suggested that 17-OHP did not have a regulatory effect on the midcycle LH peak since no positive feedback-effect on the plasma LH concentration could be observed after continuous infusion of 17-OHP in women. Swerdloff et al. (1972) reported that a subcutaneous injection of 17-OHP to the rat on the 5th day of ethinyl estradiol treatment did not result in a significant increase in serum LH, although the injection of P resulted in a significant increase in serum LH. Kobayashi et al. (1969) also reported that an injection of 17-OHP has no progesterone-like activity to facilitate ovulation in the ovulation-delayed rat.
These results are in agreement with our results that a subcutaneous injection of 17-OHP in EB primed rats has no significant facilitatory effects on the release of LH. The question arises whether this hormone is absorbed sufficiently after a single subcutaneous injection since 17-OHP is very insoluble in both oil and water. Therefore, the crystalline steroid was inserted directly into the brain.

Taleisnik et al. (1970) and Barraclough et al. (1964) reported that preoptic area (POA) was a site of the stimulatory action of P. Kalra and McCann (1975) observed that unilateral P implants into the MPO-anterior hypothalamic area (AHA) were also effective in increasing plasma LH. Kawakami et al. (1978) reported that the principal sites of the stimulatory feedback action of P on LH release were located in the DBB, the preoptic suprachiasmatic area (POSC) and AHA. Our data following the intracerebral implantation of P partially agrees with theirs.

All animals bearing 17-OHP implants or P implants in the DBB exhibited an increase in serum LH 6 hrs after implantation. In regard to implants in the MPO, all 6 rats with implants of 17-OHP, 4 of 7 with implants of P, and 4 of 6 with implants of 20-OHP exhibited an increase in serum LH. Both 17-OHP and P implantation in the SEPT failed to induce an increase in serum LH. These results suggest that the sites of stimulatory feedback action of 17-OHP on LH release are similar to those of P.

When 20 µg of E₂ in sesame oil was injected instead of EB, both 17-OHP and P implantation in the DBB as well as in the MPO failed to show marked increases in serum LH. This differential responsiveness seems to be related to the more prolonged absorption and the stronger estrogenic activity of EB as compared to E₂, because vaginal cornification was observed in 25% of rats at 48 hrs and in 100% at 72 hrs after injections of EB, as compared to 73.7% of rats at 48 hrs and 5.5% at 72 hrs after the injection of E₂. These findings suggest that the stimulatory effects of 17-OHP are synergistic with estrogen in the induction of the LH surge.

In rats, an increase of 17-OHP was observed in adrenal venous blood as well as peripheral blood during the afternoon of proestrus when the first increases in LH levels were recorded, although a peak of 17-OHP was recorded between 0200–0400 h on the day of estrus (Shaikh and Shaikh, 1979). The functional endocrine mechanisms of the human being may differ from those in the rat. But results from the present study, in conjunction with our previous observation that in some women, serum 17-OHP values increased at 8 hrs prior to, or simultaneously with, the initial midcycle rise of LH (Uemura et al., 1978), strongly suggest a physiological role for 17-OHP in inducing the LH midcycle surge in women.

References