Effect of continuous illumination on secretion of pregnane compounds by the rat ovary

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Summary. Differences in the secretion of pregnane compounds from rats with follicular polycystic ovaries under constant light and with normal preovulatory ovaries under light–dark conditions were compared. The injection of LH greatly increased the secretion of progesterone. 5α-pregnane-3,20-dione and 3α-hydroxy-5α-pregnan-20-one, in both types of ovaries, but the response of the two progesterone metabolites in the polycystic ovaries was low, suggesting low 5α-reductase activity.

Key words. Light estrous rats; polycystic ovaries; pregnane compounds.

In addition to progesterone and 20α-hydroxy-4-pregnen-3-one, the rat ovary produces and secretes two 5α-reduced derivatives of progesterone, 5α-pregnane-3,20-dione and 3α-hydroxy-5α-pregnan-20-one, and two such derivatives of 20α-hydroxy-4-pregnen-3-one, 20α-hydroxy-5α-pregnan-3-one and 5α-pregnane-3α,20α-diol [3–5]. 5α-Pregnane-3,20-dione and 3α-hydroxy-5α-pregnan-20-one are important in the regulation of follicle-stimulating hormone and luteinizing hormone (LH) secretion in immature female rats primed with pregnant mare's serum gonadotropin and maintained under constant light [6]. Sanyal and Todd [7] have shown that a large dose of 5α-pregnane-3,20-dione stimulates ovulation, as is seen by the greater number of eggs in the oviducts and the higher percentage of rats ovulating. The relationships between the physiological roles of these pregnane compounds and the secretion pattern are, however, unknown.

It is well known that androgen administration during the neonatal period or exposure to constant light (CL) causes infertility in female rats, characterized by persistent vaginal cornification and polycystic ovaries [8, 9]. It is thought that this syndrome may arise from disorders of the hypothalamus and subsequent changes in the cyclic release of LH from the pituitary gland [8]. Weisz and Lloyd [10] reported abnormal steroidogenesis in the polycystic ovaries of androgen-sterilized rats. Many disorders of rats caused by exposure to CL soon disappear when light–dark (LD) illumination is provided instead [11], so the association of steroid secretion with the tissue components of such ovaries may provide a partial answer to the question about the relationship between secretion and function. Here, we studied the relationship between the secretion of these pregnane compounds by polycystic ovaries of rats and changes in illumination.

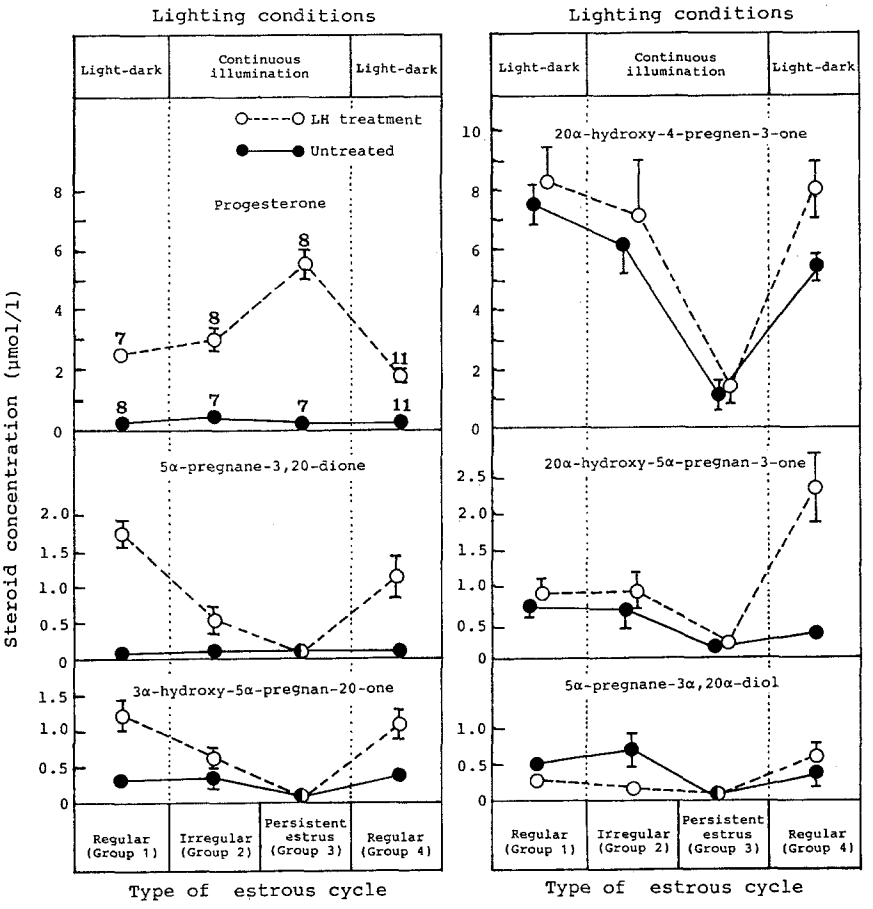
Material and methods. Animals. Sprague-Dawley rats bred in this laboratory were used. They were kept at 24 ± 1 °C and on a 14-h photoperiod (lights on from 05.00 to 19.00 h), and provided with water and a standard commercial pellet food. Vaginal smears were taken every morning. Rats that had had at least two consecutive 4-day estrous cycles by 60 days of age were moved to a room with CL, and later, some rats were placed again under LD illumination. The rats that had a cornified vagina for at least 10 consecutive days were designated as rats with persistent estrus. The animals were divided into 4 groups on the basis of the light conditions, and ovarian venous blood was collected at the follicular phase as follows: group 1, on proestrus of a 4-day estrous cycle before a change to CL; group 2, on proestrus of the irregular cycle induced by CL; group 3, on day 10 of the persistent estrus induced by CL; group 4, on proestrus of the regular estrous cycle one month after the return to LD.

Collection of ovarian venous blood. To stimulate the secretion of steroids, half of the rats in each group were injected with 2 μg of LH (NIH-LH-B7) via the external jugular vein 30 min before blood was collected. Ovarian venous blood was collected at 10.00 h under pentobarbital anesthesia [12]. A polyethylene tube (0.58 mm inner diameter and 0.97 mm outer diameter; Intermedic PE 50, Clay-Adams, Division of Becton, Dickinson and Company, NJ, USA) was used to cannulate the ovarian vein. Heparinized arterial blood was infused into the femoral vein at the rate of 10.6 ml/h during collection to maintain the blood volume. The blood used for the transfusion was obtained from the aorta of male rats. The blood collected for 30 min was centrifuged, and 2 ml of the plasma was stored at − 20 °C until used. The ovaries from which blood samples were obtained were removed immediately after blood collection and fixed in Bouin's fluid. Fixed ovaries were serially sectioned 10 μm thick and stained with hematoxylin and eosin. Sections of each ovary were examined under the microscope.

Steroid assay. The steroids were isolated by silica-gel column chromatography (SGCC) and thin-layer chromatography (TLC), and the amounts of pregnane compounds in 2 ml of ovarian venous plasma were then assayed by gas-liquid chromatography (GLC) [3, 4] and expressed as μmol/l plasma. A Shimadzu gas-chromatograph (Model GC-3AF) equipped with a hydrogen flame ionization detector and a 2-m column of 3% OV-17 on 80–100 mesh Shimalite W was used for GLC. Purification on SGCC and TLC and measurement on GLC allowed the six steroids to be assayed specifically with good accuracy and reproducibility. Results were analyzed statistically by Student's t-test. Differences between mean values with unequal variances were analyzed by the modified t-test as described by Steel and Torrie [13].

Results. Effects of illumination on vaginal smear and ovarian structure. When rats with 4-day estrous cycles under LD conditions (group 1) were moved to a room with CL, their estrous cycle became irregular (group 2) by 4–18 days (mean, 9.4 days), and finally became persistent estrus (group 3) after 22–77 days (mean, 47 days) of exposure. When rats with persistent estrus were placed again under LD conditions (group 4), the regular 4-day estrous cycle soon resumed. Ovaries of rats in groups 2 and 4 had the normal morphological appearance of those of the rats in group 1, and ovaries of rats in group 3 contained many large vesicular follicles, but no corpora lutea.

Effects of illumination on ovarian secretion of pregnane compounds. The concentrations of pregnane compounds in the ovarian venous plasma of rats after changes in illumination are shown in the figure. Progesterone and 5α-pregnane-3,20-dione were almost undetectable at all stages. The level of 20α-hydroxy-4-pregnen-3-one was high during LD (group 1), and decreased greatly during persistent estrus (group 3). When regular estrous cycles resumed after a return to LD, this steroid returned to the baseline level (group 1 vs group 4). The amounts of 3α-hydroxy-5α-pregnan-20-one, 20α-hydroxy-5α-pregnan-3-one and 5α-pregnane-3α,20α-diol were low at each stage, but the pattern of their secretion was almost the same as that for 20α-hydroxy-4-pregnen-3-one. An injection of LH before sample collection significantly ($p < 0.01$) increased the concentration of progesterone at all times tested. The progesterone level of group 3 was significantly ($p < 0.01$) higher than



Concentrations of pregnane compounds in ovarian venous plasma from rats kept in light–dark (LD), then constant light, and then LD. Dotted lines indicate rats that received LH before sample collection. Groups were compared at the follicular phase. Vertical lines indicate the standard error of the mean; the number of samples accompanies each mean value of progesterone.

that of the three other groups. The concentrations of 5α-pregane-3,20-dione and 3α-hydroxy-5α-pregnan-20-one in LH-stimulated rats were high ($p < 0.01$) in groups 1 and 4, other significant differences in these steroids were not found in any group. The concentrations of 20α-hydroxy-4-pregnen-3-one and 20α-hydroxy-5α-pregnan-3-one increased significantly ($p < 0.01$) in response to LH only in group 4. The concentration of 5α-pregnane-3α,20α-diol was unaffected by LH at all times tested.

Discussion. The changes in vaginal smears and the morphological appearance of ovaries in rats after changes in illumination resembled those reported previously[11]. Histochemical studies have shown that the site of progesterone metabolism to 20α-hydroxy-4-pregnen-3-one in the rat ovary is the involuting corpus luteum[14]. The 20α-hydroxy-4-pregnen-3-one level was high under LD conditions, but decreased after a change to CL, suggesting that 20α-hydroxysteroid dehydrogenase activity is low in the CL-induced polycystic ovary. The low level of 20α-hydroxy-4-pregnen-3-one from polycystic ovaries may be causally related to the absence of corpora lutea.

Stimulation by LH of the secretion of progesterone from the rat ovary is now an accepted fact[3–5]. In this study, the injection of LH increased the secretion of progesterone at all times tested. The ability to produce progesterone during CL was much greater than under LD conditions. Since progesterone is a precursor common to the steroid hormones, it is likely that LH increases the secretion of some of the intermediates in the biosynthetic sequence of ovarian hormones. In fact, the secretion of 5α-pregnane-3,20-dione and 3α-hydroxy-5α-pregnan-20-one was increased by LH under LD conditions. However, the ability to produce these steroids was low with CL, suggesting that the activity of 5α-reductase in the CL-induced polycystic ovary is low. The results suggest that the ability to produce high progesterone levels may be causally related to low 5α-reductase activity in the CL-induced polycystic ovaries of the rat.

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Luteolytic potency of 16-phenoxy-derivatives of prostaglandin $F_{2\alpha}$

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Summary. The binding of 16-phenoxy derivatives of prostaglandin (PG) $F_{2\alpha}$ to rat luteal membranes, and also their abortifacient potency in pregnant rats, have been studied. Competitive binding studies with various PG-analogues were performed in ovaries of juvenile rats pretreated with PMSG and HCG, and in parallel studies the abortifacient potency of these substances was tested in pregnant rats. It was observed that this class of derivatives bound to the $PGF_{2\alpha}$ receptor as well as, or even better than the parent compound $PGF_{2\alpha}$. Modifications in the carboxyl group at C-1 yielded derivatives with a higher affinity for the receptor, in decreasing order of effectiveness as follows:- COOR > COOH > OH. The data obtained from the binding studies also compared well with data on the abortifacient potency in pregnant rats. It is concluded that the addition of a phenoxy group to either the lower or upper side chain of $PGF_{2\alpha}$ may augment the binding to the receptor as well as the biological responses induced by the post receptor effect.
Key words. Prostaglandin; rat luteal membrane; receptor binding affinity; abortifacient potency; luteolytic potency.

The short duration of action of the naturally-occurring prostaglandins is a result of their fast metabolism by prostaglandin (PG)-15-OH- dehydrogenase [1,2]. One of the major goals of prostaglandin research has therefore been the generation of compounds possessing a higher affinity for the receptor molecule as well as a longer duration of action. Such a derivative should preferably retain specifically selected porperties of the natural prostaglandin. One generally used approach for the synthesis of long-acting prostaglandin is the introduction of bulky groups in the neighborhood of the 15-hydroxyl group of $PGF_{2\alpha}$ [3].

Previous experiments have shown that the introduction of such bulky groups, for example an epoxide at C-15, reduced the affinity for the receptor, whereas the addition of lipophilic substituents in the lower and upper side chain augmented binding [4]. It was then concluded that the contribution of the various substituents to the binding affinity is additive.

In this investigation, we planned to test the binding characteristics of 16-phenoxy-derivatives of $PGF_{2\alpha}$ to membrane particles isolated from superovulated rat ovaries, and if possible to correlate them with abortifacient potency in pregnant rats.

Materials and methods. Substances: (9β-^{3}H) $PGF_{2\alpha}$ (sp. act. 5.5×10^{11} Bq/mmol) was purchased from the Radiochemical Centre, Amersham. Prostaglandin derivatives were supplied by Schering AG, Berlin/Bergkamen and C-Pfizer, Inc. Groton, Connecticut. Human chorionic gonadotrophin, HCG, (2350 IU/mg) and pregnant mare serum gonadotrophin, PMSG, (2577 IU/mg) were obtained from Schering AG, Berlin and dissolved in 0.9% NaCl. Indomethacin was supplied by Boehringer, Mannheim, W. Germany. All other substances were of analytical grade.

Animals: Immature female rats (Hans-Wistar-Schering) were kept in groups of 10–12 animals, and mature rats weighing 180–200 g were kept in groups of 8–10 animals in large plastic cages. They were housed in air conditioned rooms under a controlled light regimen. The animals were provided with a standard diet of Altromin® and water ad libitum. To obtain a single well-defined generation of corpora lutea, 22-day-old female rats were injected s.c. with 50 IU PMSG, followed 72 h later by 25 IU HCG. The day of HCG administration was defined day 0 of pseudopregnancy.

Prostaglandin binding: Superovulated ovaries were homogenized with an Ultra Turrax in Tris-buffer, pH 7.4, containing 10^{-5} mol/l indomethacin. Indomethacin was added to prevent any endogenous prostaglandin synthesis [5]. Large particles and connective tissue were separated by spinning at 1000 × g for 20 min. The supernatant was filtered through a double layer of cheesecloth and the filtrate recentrifuged in a Beckman Ultracentrifuge at 105,000 × g for 60 min at 4 °C. The pellet was washed several times and resuspended to obtain a final protein concentration of approximately 1 mg/ml. Aliquots of the particulate fraction were incubated for 60 min at 37 °C with a constant amount of labeled $PGF_{2\alpha}$ (10 pmol/0.2 ml) and increasing concentrations of unlabeled $PGF_{2\alpha}$ or derivatives. Bound and free prostaglandins were separated on small Sephadex columns as described earlier [6]. The displacement of radioactively labeled ligand by non-radioactive material was generally plotted as percent binding versus the log molar concentration. Protein content of tissue preparations was determined according to Lowry [7].

Abortifacient effect of prostaglandins: Vaginal smears were examined daily at 08.00–10.00 h. When required, female Wistar rats were caged with males on the night following proestrus, and the day on which sperm was detected in the smear was designated day 1 of pregnancy. Various doses of prostaglandins or derivatives, dissolved in 0.4 ml benzylbenzoate and castor oil (1:4), were injected s.c. between days 4 and 7 of pregnancy. Plasma progesterone levels were determined on days 3, 5, 7 and 9, and autopsy was performed on day 9 of pregnancy. Animals were tested for pregnancy by counting the number of implantation scars. For each dose, groups of 5 animals were used. Abortifacient potency was then calculated by determining the smallest dose of test substance that induced luteolysis and labor in relation to $PGF_{2\alpha}$.

Radioimmunoassay for progesterone: The antibody against progesterone was raised in our laboratory by immunizing