Elucidation of the Mechanism Responsible for the Luteolytic Effect of Oestradiol During Pseudogestation in the Rat

In a recent publication (BISCHOF et al.[1]), we reported that the administration of oestradiol in the rat in very low daily doses (0.01, 0.03 and 0.1 µg/kg) during the first 6 days of pseudogestation led to a statistically significant reduction in the levels of progesterone and a significant increase in the levels of 20α OH-progesterone in ovarian venous blood withdrawn by cannulation on the 7th day, between 08.00 and 12.00 h. Doses higher than 0.1 µg/kg daily had no effect on the secretion of these substances.

The experiments described below were undertaken to elucidate the mechanism responsible for the luteolytic action exerted by these doses of oestradiol, considering the possibility of a) the participation of a uterine luteolytic factor; b) an interference with prolactine activity on the ovary; c) a modification of the pituitary functions.

Materials and methods. Adult female rats (Ivanovas, Kisslegg, Germany), weighing 200–250 g, which had been kept under standardized conditions with respect to temperature, relative humidity and lighting (light from 06.00 to 20.00 h) were mated on the evening of pro-oestrus with vasectomized males, to induce pseudogestation, the onset of which was confirmed by the presence of a vaginal plug on the morning of oestrus (day 1).

On the first day of pseudogestation, the rats were divided in 3 groups. The animals of the 1st group were left intact, those of the 2nd group were hysterectomized (after ligature of the cervical region) and those of the 3rd group hypophysectomized via the parapharyngeal approach. All treatments were begun at the latest 2 h after the operation and continued for the first 6 days of pseudogestation. Oestradiol dissolved in sesame oil was administered s.c. in a daily dose of 0.03 µg/kg.

Prolactin[2] NIH PB3 (24 IU/mg) was dissolved in 0.9% physiological saline and injected i.p. in 2 daily doses of 500 µg/kg each.

Ovine LH antiserum[3] obtained from rabbits was administered undiluted by i.p. injection in a dose of 0.1 ml, morning and evening. The controls were injected with normal rabbit serum.

On the 7th day, between 08.00 and 12.00 h, cannulae were inserted near the ovarian vein. After extraction, the blood samples were chromatographed and quantitative determinations made as described previously[1].

Results (cf. Table). a) *Possibility of a participation of a uterine luteolytic factor.* Hysterectomy on the 1st day of pseudogestation had no significant effect on the secretion of progesterone, as measured on the 7th day. By contrast, in pseudopregnant hysterectomized rats treated with 0.03 µg/kg of oestradiol daily, the rate of secretion of progesterone was reduced to 4.4 ± 1.1 µg/h, compared with 14.9 ± 2.3 µg/h in the corresponding controls. The degree of inhibition was thus similar to that observed in the intact rat. An intervention of a luteolysine of uterine origin, the secretion of which might be stimulated by oestradiol, can therefore be excluded since hysterectomy does not abolish the luteolytic effect.

b) *Possibility of an interference with prolactin activity on the ovary.* Seven days after hypophysectomy, progesterone secretion was found to be considerably diminished (1.1 ± 0.4 µg/h). Treatment with bovine prolactin partially prevented this decrease. In pseudopregnant, hypophysectomized rats treated for 6 days with the same daily dose of prolactin (1 mg/kg) together with the low dose of oestradiol, the secretion of progesterone on the 7th day was not significantly different from that found in hypophysectomized rats treated with prolactin alone. This last observation suggests that the luteolytic effect of oestradiol is not due to an inhibition of the luteotrophic activity of prolactine on the ovary.

[1] P. BISCHOF, C. KRÄHENBÜHL, P. A. DESAULLES, Experientia *30*, 200 (1974).

[2] Prolactin NIH PB 3 was generously provided by the Endocrinology Study Section, National Institute of Health, Bethesda, U.S.A.

[3] LH antiserum was kindly supplied by Dr. LOTTE SCHENKEL, to whom we are deeply indebted.

Ovarian progesterone secretion in different endocrine conditions in rats on the 7th day of pseudopregnancy

n	Operation	Treatment	Dose (kg/day)	Progesterone secretion (µg/h ± SE)	Statistical evaluation (p)	
33	Intact	Control	—	15.4 ± 2.0 (1)		
7	Hysterectomy	Control	—	14.9 ± 2.3 (2)	1–2	N.S.
9	Hysterectomy	Oestradiol	0.03 µg	4.4 ± 1.1 (3)	2–3	< 0.01
5	Hypophysectomy	Control	—	1.1 ± 0.4 (4)		
12	Hypophysectomy	Prolactine	1000 µg	8.2 ± 1.2 (5)	4–5	< 0.01
9	Hypophysectomy	Prolactine Oestradiol	1000 µg 0.03 µg	5.7 ± 1.4 (6)	5–6	N.S.
12	Intact	Oestradiol	0.03 µg	3.9 ± 0.6 (7)	1–7	< 0.001
7	Intact	Prolactine Oestradiol	1000 µg 0.03 µg	4.7 ± 1.7 (8)	7–8	N.S.
8	Intact	Normal rabbit serum Oestradiol	0.2 ml 0.03 µg	7.2 ± 1.6 (9)	7–9	N.S.
5	Intact	Anti-LHserum Oestradiol	0.2ml 0.03µg	15.1 ± 1.7 (10)	9–10	< 0.05

n, number of animals.

c) *Possibility of a modification of the pituitary functions.* The administration of oestradiol to intact rats, in a dose of 0.03 µg/kg daily on the first 6 days of pseudogestation, did not interrupt the vaginal dioestrus but resulted – as in our formerly reported experiments – in a marked inhibition of the secretion of progesterone on the 7th day (3.9 ± 0.6 as compared with 15.4 ± 2.0 µg/h). The simultaneous injection of prolactin in a dose of 1 mg/kg daily did not counteract this inhibitory effect, the secretion of progesterone in animals thus treated likewise being markedly diminished (4.7 ± 1.7 µg/h). This absence of action of prolactin under such experimental conditions suggests that oestradiol does not exert its effect on the progesterone secretion by a reduction of the prolactin levels. It must therefore be concluded that oestradiol in a low dose interefers with the secretion of another pituitary hormone. In the rats given LH antiserum together with oestradiol, we observed a significantly higher progesterone value (15.1 ± 1.7 µg/h) than in the animals which received oestradiol alone (3.9 ± 0.6 µg/h). This last observation shows that the reduction in ovarian progesterone production exerted by oestradiol has to be considered as an effect of oestrogen on the regulation of LH secretion.

Discussion and conclusion. Different publications, and especially those of BARRACLOUGH and HALLER[4], have established that the administration of low doses of oestradiol produce an increase in the secretion of LH. Repeated injections of this pituitary hormone to pseudopregnant rats exert a luteolytic effect which has been measured by morphological (ROTHCHILD[5]) and functional (YOSHINAGA, GRIEVES and SHORT[6]) criteria. The effective doses of LH are, according to ROTHCHILD[5], lower than those which induce ovulation.

Considering our observations[1] and the data in the literature, we can conclude that the drop in ovarian progesterone secretion produced by administration of low doses of oestradiol to pseudopregnant rats, is the result of a stimulation of the secretion of LH. This hypophysal hormone apparently exerts a luteolytic action, under our experimental conditions: i.e. in pseudopregnancy.

Résumé. Ce travail propose un mécanisme d'action pour l'effet lutéolytique observé chez des rats pseudogestants, traités pendant 6 jours avec une dose très faible d'oestradiol[1], cet effet lutéolytique doit être rapporté à une sécrétion accrue de LH.

P. BISCHOF, C. KRÄHENBÜHL and P. A. DESAULLES

Department of Pharmacology, Ciba-Geigy Ltd., CH-4002 Basel (Switzerland), 14 May 1974.

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[5] I. ROTHCHILD, Vit. Horm. *23*, 209 (1965).
[6] K. YOSHINAGA, S. A. GRIEVES, R. V. SHORT, J. Endocr. *38*, 423 (1967).

PRO EXPERIMENTIS

Simple Method for the Rapid Collection of Murine Blood Avoiding Death of the Animal

Estimated figures indicate that 30, 281, 783 mice were used for research purposes in 1971[1]. The reasons for this use of mice can be found in their high fertility, easy growth, and overall economy as regards procuring and maintaining. As a result there is a constant demand on the part of many laboratories to obtain blood for several purposes.

The techniques normally utilized for obtaining blood from mice are bleeding from 1. The venous plexus of the orbit,[2] 2. Intracardiac puncture,[3] 3. The inferior vena cava,[4]. Each of these methods suffers from the inconvenience that loss of animals is great. They pose special problems in certain experiments which require that multiple blood samples be collected during the course of treatment, necessitating the survival of the mice. In immunology, for example, it is important to draw blood without killing the animals, so that they can be bled again at the end of the experiment.

It is the purpose of this report to set forth a simple and rapid method by which blood can be obtained over and over again from the same mouse. No special equipment is required and the blood is taken while the animal is immobilized, thus eliminating the use of anesthetic and ensuring that the blood will be anesthetic-free. Finally, the mice do not appear to be severely stressed by the procedure.

Material and methods. 1. A plunger of a 60 ml plastic disposable syringe (A) and a plastic container (B); 2. A sterile plastic tube 5 mm in diameter and 5 cm long (C); 3. a rubber stopper with a hole 4 mm in diameter (D); 4. a graduated sterile test tube (E); 5. a 20 gauge needle (G) connected to a vacuum pump (I); 6. a 100 watts lamp (J); (Figure).

The mouse must be immobilized as shown in the Figure. The plunger may be used to adjust the plastic container to the size of the individual mouse. (It may be more

[1] *Ilar News* (Eds. R. H. YAGER and C. B. FRANK; Institute of Laboratory Animal Resources, Washington, D.C. 1972), vol. 16, p. 1.
[2] S. SCHERMER, *The Blood Morphology of Laboratory Animals*, 3rd edn. (F. A. Davis Company, Philadelphia 1967), p. 61.
[3] A. J. CRESKOFF, T. FITZ-HUGH and E. T. FABBRIS. *The Rat in Laboratory Investigation*. 2nd edn. (Eds E. T. FABBRIS and J. Q. GRIFFITH; J. B. Lippincott Co., Philadelphia 1949), p. 406.
[4] J. D. BROOME, personal communication.

C3H/HeJax mice	Weight	No. of mice	Volume of blood obtained in (ml)	Death	Sterile on culture
	26–32 g	28	0.81 ± 0.30	2	17