A Possible Process of the Secondary Sterilization: Delayed Anovulation Syndrome

A single injection of testosterone propionate (TP) to the neonatal female rat induces an anovulatory condition associated with persistent vaginal estrus[1]. It has been suggested that the influence of neonatal treatment with androgen is exerted presumably on the hypothalamus and the degree of functional alterations in the sex center depends on the dosage of androgen and the age of animals at the time of hormone injection[2-4]. A high dose of TP to the neonatal female rats effectively inhibits spontaneous ovulation postpubertally. Rats treated with low doses of TP retain the ability to ovulate for a time after puberty, eventually becoming anovulatory[5,6]. This phenomenon has been called the 'delayed anovulation syndrome' (DAS)[7] and can be assumed to occur in the female rats exposed to subphysiological levels of androgen during the critical period. A similar delayed sterility was encountered in rats which received tranquilizers, barbiturates or certain steroids concurrently with TP neonatally in order to protect against androgen sterilization[8]. In this case the protective action of these agents would be insufficient to counteract the effect of TP given neonatally. In the present study, as one step to clarify the nature of this secondary sterilizing phenomenon, the following experiments were carried out. Newborn female Sprague-Dawley rats were injected with 10 μg, 1 mg TP (in 0.05 cm^3 sesame oil) or oil vehicle and then divided into 6 treatment-groups (see Table). Ovariectomy was performed on day 30 (Groups 1, 2 and 4) or day 90 (Groups 3, 5 and 6), respectively. On day 100 ovarian fragments from immature rats were transplanted subcutaneously. All rats except 4 rats of Group 2 (sacrificed on day 200) were killed on day 140.

Androgenized rats with 10 μg TP (Group 3) showed cycles shortly after puberty and eventually developed persistent vaginal estrus before ovariectomy on day 90. Rats injected with 1 mg TP on day 5 showed persistent vaginal estrus after puberty (Group 5). The results of histological examinations on the ovarian grafts are shown in the Table. Numerous corpora lutea (CL) were recognized in the ovarian grafts of all control rats (Group 6) and in 7 out of 8 rats injected with 10 μg TP on day 5 whose own ovaries had been removed on day 30 (Group 1). However, CL were no longer found when rats receiving the same treatment as the rats of Group 1 were killed on day 200 (Group 2). In rats of Group 3 similarly androgenized with 10 μg TP in which ovariectomy had been delayed until day 90, CL were also not detected in the ovarian grafts. The grafts of these rats were characterized by the presence of many large vesicular follicles, a small old CL being found in the graft of 1 rat. Ovarian graft histology of the rats injected with 1 mg TP of Groups 4 and 5 resembled that of typical sterile polycystic ovaries, no luteinized tissue being observed.

These results could confirm earlier findings that the effect of neonatal treatment with TP on the pituitary gonadotrophic function varies with the dose[5,6]. Furthermore, in the present study, ovariectomy at prepubertal age in some way prevented androgenized rats with 10 μg TP from losing the capacity of secreting enough LH for ovulation at the time of the expected onset of anovulatory persistent estrus. This would suggest that the postpubertal influence of ovarian hormonal feedback (by estrogen and/or androgen) is participating as an important factor in the development of the DAS, as previously suggested by KIKUYAMA and KAWASHIMA[9] and GORSKI[7]. In this connection, it is of interest to note the findings of KAWASHIMA[10] that continued injections of a minute amount of estrogen (which is insufficient to induce vaginal cornification in ovariectomized rats) may induce persistent estrus syndrome. Therefore, it could be assumed that the gradual loss of the functional capacity for ovula-

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Effect of ovariectomy on the hypothalamic pituitary system in androgenized rats

Group	Treatment	No. of rats	Vaginal smear before ovariectomy	Ovarian graft[a] at autopsy (day 140 or 200)
1	TP 10 μg on day 5 ovariectomized on day 30	8	–	CL 7/8 (day 140)
2	TP 10 μg on day 5 ovariectomized on day 30	4	–	CL 0/4 (day 200)
3	TP 10 μg on day 5 ovariectomized on day 90	6	PE (or PrE)[b]	CL 0/6 (day 140)
4	TP 1 mg on day 5 ovariectomized on day 30	5	–	CL 0/5 (day 140)
5	TP 1 mg on day 5 ovariectomized on day 90	5	PE	CL 0/5 (day 140)
6	Oil on day 5 ovariectomized on day 90	5	Cyclic	CL 5/5 (day 140)

[a] Transplantation of ovarian grafts was performed on day 100. CL, corpola lutea. [b] PE, persistent vaginal estrus; PrE, prolonged estrus, 1 of 6 rats of Group 3 showed prolonged estrus.

tion in the DAS is dependent on the postpubertal ovarian activity and thought to be due to an independent modification of a neonatally partially sterilized system under the postpubertal ovarian influence. The onset of the DAS is unlikely to be ascribed to the result of the continued development of the sterilizing process initiated by androgen during the critical period. The concept proposed by SWANSON and VAN DER WERFF TEN BOSCH[6] that neonatal androgen treatment may promote the premature aging of the hypothalamic sex center would be estimated from this respect[11].

Zusammenfassung. Die Wirkung verschiedener Testosterondosen auf die postpuberale Ovulation von Ratten wurde untersucht, wobei an Ovarien der Nachweis gelang, dass der anovulatorische Zustand nach der Pubertät testosteron-dosisabhängig ist.

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Evidence for a Luteolytic Function of Prolactin in the Intact Cyclic Rat Using 2-Br-α-Ergokryptine (CB 154)

It is well known that in hypophysectomized rats corpora lutea persist for long periods. Their luteolysis can be induced by injection of prolactin (MALVEN and SAWYER[1]). This suggests that prolactin, besides its luteotropic function also has luteolytic effects in the rat under certain conditions.

Recently, prolactin has been measured using a radioimmunoassay technique in rat peripheral blood during different reproductive states (AMENOMORI et al.[2]). It was demonstrated, that prolactin rises in oestrus to concentrations higher than those observed post partum. As the cyclic rat does not have a luteal phase, the role of prolactin in the oestrus cycle is unknown. We put forward the hypothesis that prolactin may have a luteolytic function in the cyclic rat.

2-Br-α-Ergokryptine (CB 154), a derivative of the ergotoxin group of ergot alkaloids, seemed particularly suited to test this hypothesis. CB 154 exerts its main pharmacological actions on female reproduction: it interrupts pseudopregnancy in the rat, inhibits nidation and mammary carcinoma in DMBA treated female rats (HEUSON et al.[3]) and C3H/HE multiparous mice (YANAI and NAGASAWA[4]). It also depresses lactation in rabbits and sows (unpublished results). All these effects of CB 154 can be explained by its interference with the secretion of prolactin.

Material and methods. Virgin female rats of the SIV 50 strain (Ivanovas, Kisslegg, West Germany) weighing 200–250 g were used. All animals had shown at least 3 consecutive regular 4-day-cycles before the experiments. The rats were housed in a temperature (24 °C) and light (14 h/day) controlled room. They were given dietary pellets and water ad libitum.

1. *CB 154 and prolactin in intact rats.* CB 154 (3, 10 and 30 mg/kg/day in a solution of 10% ethanol) was given by stomach tube in a volume of 0.5 ml/100 g body weight. Prolactin (Ferring) was injected s.c. at a dose of 10 IU/rat/day. Treatment was begun in oestrus and continued for 3 weeks (7 days a week). Vaginal smears were taken daily. The animals were killed in oestrus and ovulation was confirmed by counting the eggs present in the oviducts. Pituitaries, uteri, adrenals and ovaries were weighed. 4–5 ovaries from each group were prepared for histological examination, sectioned serially and the total number of corpora lutea counted.

2. *CB 154 and prolactin in hypophysectomized rats.* 40 female rats were hypophysectomized in dioestrus. 8 days after the operation, ovulation was induced by injecting PMS (Gestyl, Organon Oss) 50 IU/rat s.c. and 55 h later HCG (Pregnyl, Organon Oss) 25 IU/rat s.c. 5 days after the administration of HCG the rats were divided into 4 groups, receiving CB 154 or prolaction as shown in Table II. The animals were treated for 7 days. They were killed one day after the last injection, the

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Table I. Effect of CB 154 and prolactin on ovulation, organ weights and number of corpora lutea

Group and treatment	No. of animals ovulating	Uterine weight (mg/100 g)	Adrenal weight (mg/100 g)	Pituitary weight (mg/100 g)	Ovarian weight (mg/100 g)	No. of corpora lutea per ovary
1. Controls (solvent)	10/10	158.6 ± 22.75	29.3 ± 4.26	4.51 ± 0.64	35.11 ± 7.53	21 21 27 15 = 21
2. CB 154 (3 mg/kg/day)	5/10	169.2 ± 23.56	27.7 ± 3.41	4.53 ± 0.77	39.78 ± 8.19	24 32 41 32 46 = 35
3. CB 154 (10 mg/kg/day)	9/9	137.6 ± 12.00	27.5 ± 3.27	4.48 ± 0.44	53.38 ± 7.21[a]	40 40 41 46 = 42
4. CB 154 (30 mg/kg/day)	9/10	158.2 ± 27.41	30.9 ± 5.77	4.79 ± 1.04	64.88 ± 8.97[b]	52 62 70 51 = 59
5. CB 154 (10 mg/kg/day) + prolactin (10 IU/rat/day)	4/10	159.3 ± 29.21	29.3 ± 3.79	4.37 ± 0.90	37.17 ± 4.35	20 21 23 22 = 21.5
6. CB 154 (30 mg/kg/day) + prolactin (10 IU/rat/day)	4/10	191.0 ± 44.82	30.7 ± 4.36	3.83 ± 0.61	39.78 ± 3.99	46 42 53 52 38 = 46.4
7. prolactin (10 IU/rat/day)	4/5	158.6 ± 35.35	24.9 ± 3.57	4.72 ± 1.29	34.92 ± 4.50	34 24 21 33 23 = 27

[a] $P < 0.0025$. [b] $P < 0.0005$.