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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[11] Supported by a research grant from the Ministry of Education of Japan.

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 It also depresses lactation in rabbits and sows (unpublished results).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] $P < 0.0005$.

ovaries excised, weighed and prepared for histological examination. Animals in which hypophysectomy was incomplete were discarded.

Results. 1. *CB 154 and prolactin in intact rats* (Table I). CB 154 by itself had no effect on the oestrous cycle or on ovulation. The observed slight irregularities (extension of oestrus for 1 day and delay of ovulation) in group 2, which was the last one to be treated, were also seen in our stock colony at that time. In groups 5 and 6 oestrus was extended for 1 day in most animals. When killed on the first day of oestrus, the uterus was still distended with fluid as in prooestrus. Prolactin (group 7) induced a state of dioestrus, occasionally interrupted by oestrus.

No differences in the weights of pituitary, adrenal or uterus were observed. The increase in uterine weight in group 6 can probably be accounted for by increased water content due to delayed ovulation.

A dose-dependent increase in ovarian weight and in the number of corpora lutea was found in rats treated with CB 154. 3 mg/kg seems to be a threshold dose: it increased the number of corpora lutea in $^2/_5$ ovaries.

Prolactin, 10 IU/rat/day reversed the effect of CB 154 totally (10 mg/kg) or partially (30 mg/kg).

Corpora lutea in CB 154 treated rats were not, or only slightly enlarged, but failed to undergo normal involution. The luteal cells appeared healthy, but were not functional as judged from the vaginal smear. The age of the corpora lutea could not be determined.

Ovaries from rats receiving CB 154 and prolactin could not be distinguished from those of control animals. In group 7 (prolactin) luteolysis was more marked than in controls. This was particularly evident in the newly formed corpora lutea.

2. *CB 154 and prolactin in hypophysectomized rats* (Table II). The ovarian weight of group II and III, receiving prolactin and prolactin + CB 154 respectively, was about 20 mg less than in group I and IV (controls and CB 154). Prolactin induced a marked luteolysis which could not be overcome by CB 154. CB 154 itself had no effect on ovarian weight or histological appearance of corpora lutea.

Table II. Effect of CB 154 on the luteolysis induced by prolactin in hypophysectomized rats

Group and treatment	*n*	Weight of ovaries (mg/100 g)	*P*
I. Controls	9	53.27 ± 14.17	
II. Prolactin (10 IU/rat/day) s.c.	9	33.72 ± 7.19	< 0.005[a]
III. Prolactin (10 IU/rat/day) s.c. + CB 154 (10 mg/kg/day) orally	10	32.39 ± 7.75	< 0.0025[a]
IV. CB 154 (10 mg/kg/day) orally	8	55.18 ± 6.00	n.s.

[a] No significant difference between groups II and III. *n* = number of animals.

Discussion. The enlargement of ovaries and accumulation of corpora lutea in rats treated with crude ergotoxin, a mixture of ergot alkaloids, was first described in 1944 by FITZHUGH et al.[5]. Ergocornin, a component of ergotoxin (KISCH[6]) and 2-Br-α-Ergokryptine (HEUSON et al.[3]) were reported to have the same effect.

Our results are consistent with those of FITZHUGH and of KISCH, and confirm those of HEUSON. Furthermore we were able to show, that simultaneous administration of prolactin counteracted the effect of CB 154. As CB 154 itself has no luteotropic action (results of experiment 2) this implies that CB 154 interferes with endogenous prolactin. This interference does not occur at the ovarian level, but is mediated by the pituitary. Whether CB 154 acts at the hypothalamic or hypophyseal level cannot yet be concluded, although results of ZEILMAKER and CARLSEN[7] and of PASTEELS[8] point to the pituitary as the site of action.

The present results demonstrate that blocking the secretion of prolactin during the oestrous cycle leads to an accumulation of corpora lutea which fail to undergo normal involution. It is concluded that prolactin is the major luteolytic factor in the cyclic rat.

The dual function of prolactin explains the paradoxical fact, that CB 154 induces involution of corpora lutea in pregnant rats, thereby preventing nidation, while maintaining and accumulating corpora lutea in cyclic rats. Whether prolactin exerts a luteotropic or luteolytic effect depends on the time of administration after corpus luteum formation (MALVEN[9]). Why the newly formed corpora lutea in a cyclic rat respond to prolactin with morphological regression instead of being transformed into a functional state is an intriguing question awaiting further investigation.

Zusammenfassung. Mit Hilfe von 2-Br-α-Ergokryptin (CB 154) konnte bei intakten weiblichen Ratten mit normalem Zyklus gezeigt werden, dass Hemmung der Prolactinsekretion die Lyse der corpora lutea ovulationis verhindert.

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Autoradiographic Localization of Radioactivity in Female Rat Neocortex After Injection of Tritiated Estradiol

During the autoradiographic study of developmental changes in the uptake of radioactivity by the hypothalamo-hypophysial system following a single injection of tritiated estradiol in the female rat (PRESL et al.[1]), an unexpected incorporation of radioactivity into the neocortex has been demonstrated.

Intact female Wistar rats were injected i.p. at the age of 5, 10, 15, 20, 25, 30 and 50 days with estradiol-