Inhibition of Spontaneous and Induced Ovulation in Rats by Non-Steroidal Agents[1]

Ovulation induced in juvenile rats by PMS is often used instead of the spontaneous ovulation in adult rats as an experimental model for physiological and pharmacological studies[2]. The question should be asked, whether it is correct to regard the two models as equivalent, e.g. in the assessment of the ovulation inhibitory potency of non-steroidal drugs. BROWN[3] reported that methysergide and LSD, two potent serotonin antagonists, are very active in inhibiting PMS induced ovulation in juvenile mice but quite inactive as inhibitors of spontaneous ovulation in adult mice. HÖKFELT and FUXE[4] described that 2-Br-α-ergokryptine (CB 154) and ergocornine inhibit induced ovulation in juvenile rats. These two compounds are widely used as experimental tools to inhibit prolactin secretion. Ergocornine has been known also to inhibit spontaneous ovulation in adult rats[5], but for CB 154 given to adult rats we observed in unpublished experiments that the ovulation inhibitory action only occurs at doses higher than those used by HÖKFELT and FUXE. The two ovulatory models do not seem to produce comparable results and it seemed therefore necessary to compare the two models in a quantitative way by using drugs of different chemical classes, that are known to interfere with the processes of gonadotropic hormone release in rats.

Material and methods. Adult female rats (200–250 g) of the Ivanovas-Wistar strain (Kisslegg, West Germany) were used in our experiments. The animals were kept under standard conditions: 14 h light (from 04.00 to 18.00); 24 °C; 55–60% rel. humidity; food and water ad libitum. Animals with regular 4-day cycles were injected s.c. in proestrus at noon with chlorpromazine-hydrochloride (CPZ), phenobarbital-Na (Phen), ergocornine-methanesulfonate (ECO), or 2-Br-α-ergokryptine-mesilate (CB 154). Controls were injected with solvent. In oestrus at 09.00 h the animals were killed, the tubae dissected free and the ova counted with the aid of a dissecting microscope.

Juvenile 24-day-old rats of the same strain were injected with 15 IU or with 5 IU PMS (pregnant mare serum, Organon) and 51 h later with either of the 4 substances mentioned above. Controls received solvent. On the following morning at 09.00 h the animals were killed, dissected and the ova counted as mentioned for the adults.

For the evaluation of the experiments the ovulation rate in an experimental group was used, ovulation being considered as having occurred if one or more eggs were counted and ovulation was considered to be inhibited only if no egg was found. ED_{50} values for suppression of ovulation were calculated using the method of LITCHFIELD and WILCOXON[6].

In order to acquire some information on the hormonal situation of the two experimental models serum LH and prolactin values were measured also in some of the experiments. LH was measured by a radioimmunoassay described elsewhere[7]. For prolactin measurements an assay system similar in sensitivity and specificity to the NIH-radioimmunoassay kit was used. Results are expressed in LH-NIH-RP1 and prolactin NIH-RP1[8]. In all experiments blood was collected by decapitation.

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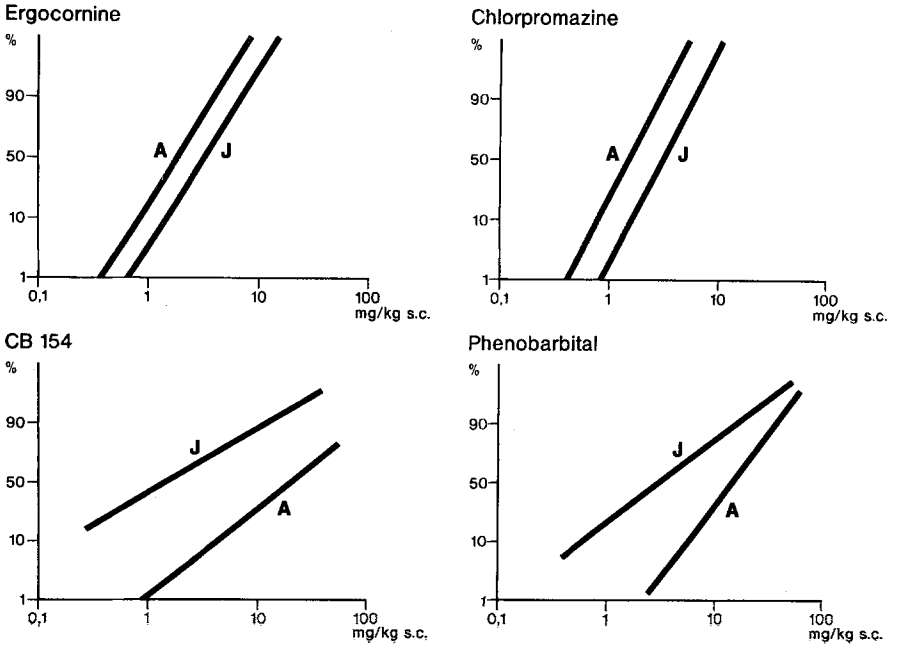

Suppression of the ovulation, dose-response curves A, adult; J, juvenile.

Table I. Effect of different doses of PMS on ovulation in immature rats in comparison to spontaneously ovulating adult rats

	Number of ova/ovulating rat (means ± SEM)	(range)	Ovulation rate
Spontaneous ovulation (adults)	13.5 ± 0.8	10–18	10/10
Induced ovulation (juveniles)			
PMS, 15 U	28.4 ± 3.74	3–46	13/15
PMS, 5 U	10.6 ± 1.37	4–18	9/14

Treatment schedules. Adults: proestrus noon: 0.9% NaCl s.c.; estrus, 09.00 h killed. Juveniles: day 24, 09.00 h PMS s.c., day 26, noon 0.9% NaCl s.c.; day 27, 09.00 h killed.

Table II. Serum-LH and prolactin in juvenile and adult rats (means ± SEM)

	Serum LH (ng/ml)		Serum prolactin (ng/ml)	
Adult ($n = 5$)	Proestrus 18.00 h 934 ± 114	Estrus 09.00 h 33.6 ± 1.56[a]	Proestrus 18.00 h 102.6 ± 25.2	Estrus 09.00 h 12.7 ± 2.41[a]
Juvenile PMS 15 U, day 24 ($n = 10$)	495 ± 184	308 ± 106 n.s.	27.5 ± 7.59	39.5 ± 6.0 n.s.
Juvenile PMS 5 U day 24 ($n = 10$)	209 ± 60	25.8 ± 0.69[a]	36.6 ± 17.7	52.0 ± 16.4 n.s.

n.s., $p > 0.05$; [a] $p < 0.01$ (difference between 18.00 h and 09.00 h). For juvenile rats: proestrus 18.00 h = day 26, 18.00 h; estrus 09.00 h = day 27, 09.00 h.

Results. Administration of 15 U of PMS to juvenile rats caused superovulation in comparison to the number of ova shed by spontaneously ovulating adult rats. Injection of 5 U PMS induced release of nearly a normal number of ova counted in the oviduct of rats which ovulated, although the ovulation rate in these animals was lower than in the adult ones (Table I). In both juvenile groups there is more variation in the number of ova per rat than in the adult group.

After treating immature rats with 5 U or 15 U PMS on the 24th day of age we measured the serum LH and prolactin levels 51 or 72 h later, times which correspond to 18.00 h proestrus and 09.00 h oestrus in adult rats. These results are presented in Table II. While high values of serum LH and prolactin could be found in the adult rats on the proestrus afternoon, no such elevated values for prolactin were observed in the juvenile rats at the corresponding hours. After 15 U PMS we found unexpected high LH-levels on the morning following ovulation.

Table III. Inhibition of the ovulatory process in rats

	Ovulation inhibition Adult, spontaneous (ED_{50})	Juvenile, PMS induced[a] (ED_{50})
Ergocornine	1.7 (1.2–2.4)	3.2 (2.2–7.0)
Chlorpromazine	1.5 (1.1–2.1)	3.0 (2.2–4.1)
CB 154	20 (13.7–29.0)	1.5 (0.5–5.9)
Phenobarbital	14 (2.9–21.1)	3.8 (2.2–6.4)

( ), 95% confidence limits; ED_{50} mg/kg s.c. [a] PMS, 15 I.U. per rat.

In Table III, the ED_{50}-values calculated from the dose-response studies with 4 different drugs are presented. ECO and CPZ are similarly active in both models, whereas the ED_{50}-values of CB 154 and Phen are each clearly different for the two models. Their activities in inhibiting ovulation are several times weaker in the adult than in the juvenile rats. Comparing the dose-response curves of the Figure, it can be seen that the slopes for ECO and CPZ are similar in both models, in contrast the dose-response curves for CB 154 and Phen differ in the juvenile and adult model.

In Table IV, serum LH and prolactin levels of adult control animals and CB 154 treated adult rats are shown. Prolactin but not LH is greatly reduced after a 1.5 mg/kg dose in comparison to the controls. In Table V, the results of a similar experiment in juvenile rats are shown: LH and prolactin were found significantly reduced after 1.5 mg/kg CB 154.

Discussion. WILSON et al.[9] have published an excellent analysis of the hormonal situation of the 30-day-old PMS-treated Wistar rat, showing that their LH levels around the time of ovulation are very similar to those in adult rats. Our results indicate that both in physiological aspects and as a pharmacological model the 24-day-old PMS-treated rat is different from the adult rat of the same strain. We observed that at 18.00 h in the evening before ovulation the serum LH levels are much lower in the PMS-treated juvenile rats than in the adult rats. Also the variability of the number of ova shed during that night is enormous. Although 5 U PMS instead of 15 U PMS will produce numbers of ova similar to the number in

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Table IV. Serum LH and prolactin in adult rats on proestrus day at 18.00 h treated with CB 154 at noon

	CB 154 (mg/kg s.c.)				
	0	1.5	10	20	100
LH (ng/ml)	1098 ± 84	1020 ± 279 n.s.	267 ± 87[b]	225 ± 124[b]	371 ± 180[a]
Prolactin (ng/ml)	114 ± 11	9.0 ± 2.4[b]	2.9 ± 0.41[b]	2.8 ± 0.28[b]	3.0 ± 0.22[b]

(means ± SEM, $n = 10$) n.s. $p > 0.05$; [a] $p < 0.05$; [b] $p < 0.001$.

Table V. Serum LH and prolactin in juvenile rats

	CB 154 (mg/kg s.c.)			
	0	1.5	5	10
LH (ng/ml)	209 ± 60	34 ± 0.98[a]	31 ± 0.91[a]	32 ± 1.29[a]
Prolactin (ng/ml)	36.6 ± 7.6	7.1 ± 1.64[a]	4.6 ± 0.5[b]	3.0 ± 0.44[b]

Treatment schedule. Day 24, 09.00 h: PMS 5 U, s.c.; day 26, noon: CB 154; day 26, 18.00 h: killed. Means ± SEM, $n = 10$. [a] $p < 0.01$; [b] $p < 0.001$.

adults, the number of animals ovulating is reduced. Furthermore we could not find the expected increased preovulatory prolactin level in the PMS-treated juvenile rats; instead we observed rather higher levels in the morning after ovulation than in the adult rats. Therefore, the juvenile rats treated on day 24 with PMS seem to be in a different hormonal situation than the spontaneously ovulating adult rat.

The difference in the hormonal situation between the two groups of rats may manifest itself also functionally when such animals are used for pharmacological manipulation of the ovulatory process. Such drugs either act qualitatively and quantitatively similar in both models, i.e. ECO and CPZ or they produce strikingly different results, i.e. CB 154 and phenobarbital.

It is these two compounds that bring to light the different situation existing in the juvenile PMS-treated rats and the adult rats as to the ovulatory mechanisms. For phenobarbital which is said to interfere witth steroid synthesis[10] one might speculate that a difference in central sensitivity for steroid feed-back[11] in the adult and the juvenile rats bring about the greater ovulation inhibitory activity of phenobarbital in the latter model.

For CB 154 we are not aware of enough information elements to produce a good hypothesis. The difference in sensitivity of adult and juvenile rats to CB 154 is clearly one of the LH secretory mechanism but not of the prolactin secretion. It has recently been shown[12] that 5 mg/kg s.c. CB 154 only partially suppressed LH release induced by LH-RF in 8-week-old rats spayed at the age of 4 weeks and pretreated 3 days before the experiment with oestradiol plus progesterone. This finding shows that in rats which have been exposed to ovarian steroids CB 154 exerts only a weak LH release inhibitory effect. Thus one could speculate that the difference in activity of CB 154 as an ovulation inhibitor in adult and juvenile rats could be traced back to a difference in the hormonal climate existing in the two age groups.

As mentioned in the introduction, BROWN[3] observed that methysergide and LSD inhibited ovulation in PMS-treated juvenile but not in adult mice. Because BROWN used 2 different strains of mice for the two ovulation models it cannot be decided whether the difference in activity of the 2 alkaloids is due to the strain difference or is another example of the phenomenon observed in rats with Phen and with CB 154.

The question asked in the introduction, whether the ovulation processes in PMS-pretreated juvenile and in adult rats are two equivalent models for the assessment of potentially ovulation inhibitory non-steroid drugs must be answered in the negative. Typically CB 154 which in juvenile PMS-treated rats suppresses preovulatory LH in the same doses that are necessary to suppress prolactin is successfully used clinically for the treatment of galactorrhoea-amenorrhoea in women and for galactorrhoea-hypogonadism in male patients[13]. Also it has been found that the continuous treatment of female volunteers with CB 154 fails to influence the mid-cycle LH peak[14].

Zusammenfassung. Die beiden häufig als gleichwertig betrachteten Ovulationsmodelle, die PMS induzierte Ovulation juveniler Ratten und die spontane Ovulation adulter Ratten, wurden nach physiologischen und pharmakologischen Kriterien miteinander verglichen. Es wird nachgewiesen, dass die beiden Modelle physiologisch nicht gleichartige endokrine Bedingungen widerspiegeln und dass sie pharmakologisch unterschiedliche Aussagen ergeben.

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