following withdrawal. The type of convulsive seizures observed also seemed to be influenced by 6-HODA pretreatment since nearly equal numbers of wild runs, clonic and tonic seizures were observed in these animals while clonic seizures were by far the most common type observed in saline pretreated rats.

Earlier studies have also demonstrated close correlations between convulsive seizure susceptibility and low brain noradrenaline. SCHLESINGER and BOGGAN[11] showed a correlation between age dependent audiogenic seizure susceptibility and brain noradrenaline content. REITER and MORGAN[12] demonstrated a close correlation between the convulsion susceptibility of parathyroidectomized rats following subsequent pinealectomy and a decrease in brain noradrenaline. ARNOLD, RACINE and WISE[13] demonstrated an increase in sensitivity to electrically induced convulsive seizures following noradrenaline depletion with 6-HODA.

Those rats which were first pretreated with 6-HODA and then treated chronically with barbital had significantly lower body weights than either control rats or rats pretreated with saline and then given barbital. Previous investigators have shown that the intraventricular administration of 6-HODA results in a decrease in food consumption and a lower body weight[14]. In this study rats which received only 6-HODA pretreatment did not have significantly lower body weights than controls, but this may be related to the very low sample size of this particular group. In this study chronic barbital treatment alone resulted in significantly higher body weights. Further studies will be required to determine if this is a consistent finding and if it is secondary to a stimulation of food consumption. The present data show that the depletion of brain noradrenaline and dopamine causes a markedly earlier onset and a somewhat greater incidence of spontaneous seizures following the withdrawal of barbital dependent rats. From the present data, it is not possible to determine which of these 2 catecholamines is more important in producing this effect. On the other hand, it remains to be shown that a decrease in the activity of either a noradrenaline or a dopamine pathway in the brain is responsible for the spontaneous convulsive seizures observed following barbital withdrawal.

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Inhibition of Ovulation in Rats by Antagonists to Serotonin and by a New Tricyclic Compound

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Summary. The ovulation inhibiting activity in adult rats of the 5HT-antagonists cyproheptadine, mianserin and methysergide is shown. Furthermore the activity of a newly synthetized Cycloheptathiophenederivative, compound 26–921, which inhibits LH-secretion and consequently ovulation, is described.

In recent years, a considerable amount of information has been obtained concerning the neuroendocrine control of anterior pituitary function. It is assumed that changes in amine metabolism in discrete parts of the brain influence the secretion of gonadotropines and consequently the process of ovulation[1]. The effect of pharmacologically-induced changes in adrenergic transmission have strengthened this assumption[2]. Relatively little work has been done towards analyzing the role of serotonin (5HT) in the control of ovulation. BROWN[3] reported that serotonin antagonists such as LSD and methysergide[4,5] inhibit PMS-induced ovulation in immature mice. As we have observed profound differences between the pharmacological responsiveness of PMS-induced ovulation as compared to spontaneous ovulation[6], it seemed necessary to investigate whether some well known 5HT-antagonists are active in spontaneously ovulating adult rats also.

Here we wish to report on the ovulation inhibiting activity of these 5HT-antagonists: besides methysergide, an ergot derivative, compounds of different chemical classes were included. Further we describe the activity of a newly synthetized cycloheptathiophene-derivative, compound 26–921[7].

Material and methods. Adult female rats of the Ivanovas Wistar strain (200–250 g) were used in our experiments. The conditions of experimentation were the same as described recently[6]. The animals were injected s.c. in prooestrus at noon with the following compounds: Compound 26–921, 9,10-dihydro-10-methyl-4-(1-methyl-4-piperidyliden)-4-H-*benzo* (4,5) *cyclohepta* (1,2-b) *thiophen*-hydrogenmalate, dissolved in saline; methysergide-hydrogenmalate, dissolved in alcohol and tartaric acid; cyproheptadine-hydrochloride (MS&D), and mianserin-hydrochloride (Organon), both dissolved in saline. In oestrus at 09.00 h the rats were sacrificed and ova were counted in both Fallopian tubes with the aid of a dissecting microscope. Only when no eggs were found, was ovulation considered to be inhibited. Mean number of eggs per ovulating rat in each treatment group was calculated. From the proportion of rats ovulating in any treatment group the 50% inhibitory dose (ED_{50}) was calculated using the method of LITCHFIELD and WILCOXON[8]. In another series of experiments, compound 26–921 0.3 mg/kg s.c. was injected in prooestrus-rats at noon, controls receiving 0.9% saline solution. The animals were decapitated either at 18.00 h of the same day, or at

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08.00 h the following norning in oestrus. Furthermore control prooestrus-rats were sacrificed at 08.00 h and 14.00 h also.

LH was measured in serum and in the adenohypophyses of each rat by radioimmunoassay[9], and the results are expressed in ng/ml, or μg/mg in terms of LH-NIH-RP1. Statistical analysis was performed with the Student's *t*-test.

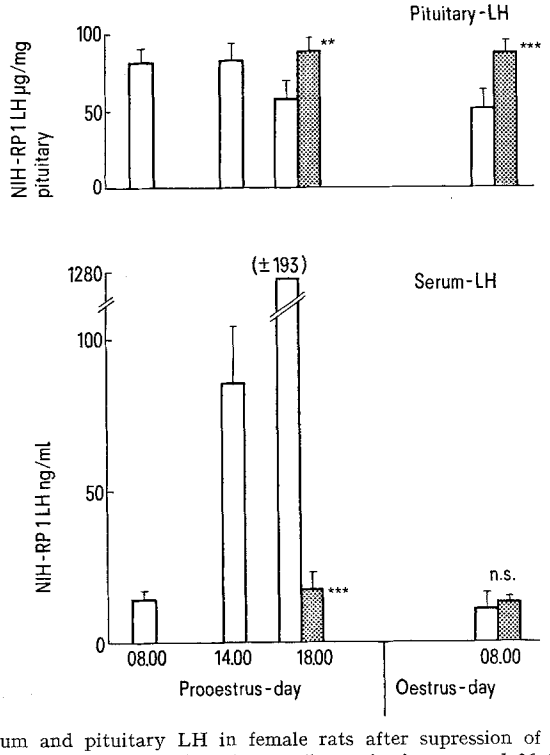
Table I. Effect of 5HT- antagonists on ovulation in rats

Compound mg/kg s.c., ($n = 5$)	Ovulation inhibition (%) 1	0.5
Methysergide	60	0
Mianserin	100	100
Cyproheptadine	100	100

Table II. Effect of compound 26-921 on ovulation in adult rats

Treatment (mg/kg s.c.)	Ovulation rate	Ovulation (%)	No. of ova/ovulating rat (means ± SE)
0.3	0/10	0	0
0.2	4/10	40	11.5 ± 0.64
0.1	3/ 9	33	13.0 ± 2.51
0.05	4/10	40	12.0 ± 0.75
0.03	6/10	60	11.8 ± 0.87
0.02	7/10	70	14.4 ± 0.64
0.01	10/10	100	12.4 ± 0.47
Controls	10/10	100	13.5 ± 0.80

Treatment schedule: Prooestrus noon: compound 26-921 or 0.9% saline s.c. Oestrus 09.00 h: killed.



Serum and pituitary LH in female rats after supression of LH secretion by a single dose (0.3 mg/kg s.c.) of compound 26–921, treated in prooestrus at noon. White columns: controls, black columns: 26–921-treated rats. (means ± SE of 10 animals). n.s. $p > 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

Results. The ovulation inhibitory action of some known specific serotonin antagonists at 1 and 0.5 mg/kg s.c. was observed and the results given in Table I. Mianserin and cyproheptadine were both more potent than methysergide in inhibiting ovulation in rats. Inhibition of spontaneous ovulation was produced by compound 26–921 in doses above 10 μg/kg s.c. (Table II) with an ED_{50} = 0.06 mg/kg s.c. (95% confidence limits 0.03–0.1 mg/kg). The effect on ovulation is of the all-or-none type, i.e. the number of ova per rat ovulating is not reduced, but the proportion of ovulating rats per treatment group diminishes with increasing doses of the compound.

The ovulation inhibitory action of compound 26–921 is based on its ability in rats to prevent the preovulatory LH surge. In the Figure this is demonstrated using 0.3 mg/kg s.c. At 18.00 h of the prooestrus day the serum LH level of treated rats (black columns) is significantly lower, than that of the controls. Concomitantly the LH content of the pituitaries of treated animals is significantly higher than in controls. This accumulation of LH in the pituitaries after treatment with compound 26–921 can still be observed the following morning (at oestrus) at 08.00 h.

Discussion. BROWN[3] had shown that methysergide and LSD inhibit PMS-induced ovulation in mice. Our study shows that this effect is also observed in the spontaneously ovulating adult rat. This effect is not restricted to ergot derivatives, but is also found with 5HT-antagonists of a very different chemical class[10,11]. Furthermore, we found that certain derivatives of cycloheptathiophene also inhibit ovulation in adults rat. One of these derivatives, compound 26–921, has now been shown to inhibit in the rat increased LH secretion in the preovulatory phase. This action of serotonin antagonists is not necessarily restricted to the rat. It has recently been observed[12] that patients with carcinoid syndrome show increased serum LH levels which were reduced by treatment with cyproheptadine.

The primary mode of action of the drugs used or mentioned in this study is assumed to be serotonin receptor blockade at one step in the hypothalamic events that control preovulatory LH release. This unitary concept may prove to be too simple to explain the ovulation inhibitory action of both the cyproheptadinelike drugs and the ergot derivatives. It has recently been observed that another serotonin antagonist[13] and ovulation inhibitor[6], ergocornine, also acts as a CNS serotonin receptor stimulator[14]. Obviously detailed studied are needed to analyze differences in the mode of action of serotonin receptor antagonists of ergot and non-ergot compounds on the control of ganodotropin secretion.

Experiments are in progress to analyse the site and mode of action of compound 26–921.

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