The Effect of Clomiphene and Cyclofenil upon Pituitary LH and Hypothalamic LH-Releasing-Factor Content in the Female Rat

Clomiphene (1-*p*-(β-diethylaminoethoxy)-phenyl-1, 2-diphenyl-2-chlorethylene) has been shown to be a potent stimulator of ovulation in the anovulatory woman and also in the female rat, which has been made anovulatory by induction of pseudopregnancy or persistent oestrous[1, 2]. Clomiphene citrate[3] was found to inhibit the binding of radioactive oestradiol by slices of human myometrium and rabbit uteri, and also to inhibit the binding of oestradiol to specific receptor proteins in the soluble fraction of uterine homogenates from rabbit uteri[4]. As only $^1/_{100}$ of the dose of clomiphene was needed to prevent the uptake of 6, 7-^{3}H-oestradiol by the pituitary as compared to the hypothalamus, it was proposed by KATO et al.[5] that the pituitary gland was the primary site of action. An inhibitory effect of clomiphene upon progesterone synthesis in human corpus luteum slices incubated in vitro was described by HAMMERSTEIN[6], and considered to be an indication of the ovary being the target organ of clomiphene action. Recent studies indicated, however, a hypothalamic effect of clomiphene, as i.v. applied clomiphene was shown to induce a dose-dependent decrease of hypothalamic FSH-RF and a concomitant increase of plasma FSH[7].

The present study was concerned with the effects of clomiphene upon the hypothalamic content of LH-RF and the pituitary content of LH. Since cyclofenil[8] (*Bis*-(*p*-acetoxyphenyl)-cyclohexilidene-methane) was found to affect plasma FSH and hypothalamic FSH-RF in the same manner[9], the effect of this compound was also studied.

Materials and methods. Female Wistar rats of 180–200 g body weight kept under standard conditions were oophorectomized 4 weeks prior to the injection of the test compounds. To assure certainty of vaginal anoestrous, vaginal smears were performed during the last 7 days prior to the experiments. On day 3 and 2 before the injection of the compounds, the pituitaries were blocked by injection of 50 µg of oestradiol and 25 mg of progesterone s.c. per day.

Clomiphene citrate was used as an aqueous solution. Cyclofenil was dissolved in propylene glycole as described before[9]. The dissolved test substances were injected into a tail vein. 5 animals were used per dose level. Doses are expressed as the weight of the compounds administered to each rat.

Following the injection of cyclofenil, the animals were sacrificed after 30 min. When clomiphene was used, the assays were performed after 2 h, since at that time the effect was found to be more pronounced than after 30 min.

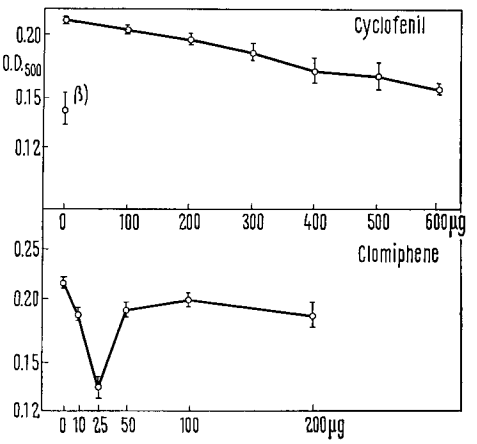
After sacrifice the pituitaries and hypothalami were removed at once. The latter were immediately extracted, while the pituitaries were placed in acetone and stored until further processing at −18 °C. Hypothalmic extracts were prepared according to the method described by RAMIREZ et al.[10].

The pituitary LH content was measured by a modification of the method described by PARLOW[11], the details of which have been reported elsewhere[12]. Each assay rat received 0.15 mg of pituitary powder suspended in 1.5 ml of Ringer's solution i.v. The LH-RF content of the hypothalamus was determined by the method of RAMIREZ et al.[10]. The pooled hypothalamic extracts were injected into groups of 3–4 recipient rats, which had been spayed and blocked with oestrogen and progesterone. LH-RF was measured as a function of the pituitary LH-depletion in the recipient rats.

A solvent-treated group comprising 6 animals, and a second group the animals of which were treated with 2 IU HCG[13], were run with each experiment. The values are given as group means ± SEM[14]. The significance of differences was calculated by Student's *t*-test, or by analysis of variance.

A standard curve using HCG had been established, which proved the assay to be linear on a semi-log scale in the range used in this study.

Results and discussion. The results of the present study are summarized in the Figure and in the Table. There was no significant change of pituitary LH as compared with untreated controls when 10 µg/rat of clomiphene were injected, but the application of 25 µg/rat was followed by a significant decrease ($p < 0.01$). When the dose was raised to 50 µg/rat, the pituitary LH content was found to be higher and to equal the level found after administration of 10 µg/rat. No change was seen after further increases of dosage to 100 and 200 µg/rat, respectively. Similar changes were



Effect of clomiphene citrate and of cyclofenil upon pituitary LH in oophorectomized, oestrogen-progesterone blocked rats. LH was measured by the method of PARLOW[11]. Each point represents the mean value of 5 animals. Vertical bars denote standard error of the mean. A fall of O.D.$_{500}$ represents a fall in pituitary LH. For further details of experimental technique refer to text. β), mean pituitary LH ± SEM of oophorectomized, unblocked rats.

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found when the hypothalamic LH-RF was determined, which also showed a significant decrease ($p < 0.05$) at the dose of 25 µg/rat.

A more pronounced effect was seen when cyclofenil was used. This compound was injected in doses ranging from 100 to 600 µg/rat. It was found that the injection of increasing amounts of this compound resulted in a straight-lined fall (semi-log scale) of both pituitary LH and of hypothalamic LH-RF content. An analysis of variance revealed the decrease of both pituitary LH ($P < 0.01$, $N = 7$) and of hypothalamic LH-RF ($P < 0.05$, $N = 5$) to be significant.

The effect of clomiphene citrate and of cyclofenil upon hypothalamic LH-RF content in the oophorectomized, oestrogen-progesterone blocked rat

Treatment	Dose (µg/rat)	No. of rats	$O.D._{500}$ (mean ± SEM)
Clomiphene citrate	0	5	0.144 ± 0.007
	10	5	0.132 ± 0.014
	25	5	0.159 ± 0.002[a]
	50	5	0.115 ± 0.006[a]
Cyclofenil	0	6	0.171 ± 0.011
	100	6	0.180 ± 0.016
	300	6	0.193 ± 0.013
	500	6	0.214 ± 0.007
Castrated, unblocked animals	0	6	0.230 ± 0.014

[a] Significant against solvent control ($p < 0.05$). LH was measured according to PARLOW[11], LH-RF by the method of RAMIREZ et al.[10]. The pooled hypothalamic extracts were injected into 3 recipient rats/dose and their pituitary LH content was determined. A rise of $O.D._{500}$ indicates a fall in hypothalamic LH-RF. For experimental details refer to text. An analysis of variance of the results obtained with cyclofenil revealed significance at the 5% level.

The results of this study show unequivocally that both ovulation inducers tested affect pituitary LH and hypothalamic LH-RF in the experimental model used. It was also found that the actions of clomiphene and of cyclofenil upon LH and LH-RF differed considerably from the effects previously observed on FSH and FSH-RF[7,9]. This indicates clearly that the modes of action of these compounds in respect to LH-release are not identical when tested under the present experimental conditions.

Zusammenfassung. Der Effekt von Clomifenhydrogencitrat und von Cyclofenil auf den LH-Gehalt der Hypophyse und den LH-RF-Gehalt des Hypothalamus wurde an kastrierten, weiblichen Ratten untersucht, die mit Östradiol und Progesteron blockiert worden waren. Während die Injektion von Cyclofenil im Dosisbereich von 100 bis 600 µg/Tier zu einem Abfall von sowohl LH als auch LH-RF führte, konnte bei Verabreichung von Clomifen nur bei der Gabe von 25 µg/Tier eine signifikante Herabsetzung von LH und LH-RF beobachtet werden.

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Vitellogenesis in Bidderian Oocytes After Diethylstilbestrol Dipropionate Treatment on *Bufo bufo* Adult Males

The problems about sexuality of *Bufonidae* are complicated by the presence, near the normal gonad, of a characteristic structure: the Bidder's organ, which in early larval stages always develops as a rudimental ovary, both in the male and female genotypes, and which persists in all the adult males and in some adult females.

Bidder's organ oogenesis has to be referred to as 'abortive' because the diplotenic oocytes, unable to accumulate yolk, degenerate at the end of the II growth period (previtellogenesis)[1]. Only after testis ablation, the Bidder's organ becomes an active ovary in the space of 2–7 years[2].

The aim of this paper is to present preliminary results of research work in which we have approached the problem of endocrine factors concerning the abortive bidderian oogenesis.

Material and methods. In March 1968 we administered a synthetic oestrogen, the diethylstilbestrol dipropionate (DSD), on *Bufo bufo* adult males in form of subcutaneous implant of a 25 mg solid tablet. We have chosen this way of administration of DSD because the tablet is very slowly adsorbed in the period of several months.

According to the indications on the bidderian oogenesis obtained in our precedent research work[3], we have killed some specimens of control and treated groups, 1, 5, 9 months after the administration of synthetic oestrogen.

Results and discussion. The histological study of testes proves that, until the end of the experiment, both in the control and in the DSD treated animals, the spermatogenesis goes on normally, since we have always found all the stages of spermatogenesis (spermatogonia, spermatocytes, spermatides, spermatozoa).

On the contrary, the histological study of Bidder's organs has given some interesting differences between the control animals and the treated ones.

In spring, Bidder's organs, both in the control and in the treated group, show a lot of diplotenic oocytes, provided whith lampbrush chromosomes, engaged in the previtellogenetic II growth period; more increased oocytes, unable to accumulate yolk, as usually, degenerate.

In summer, the Bidder's organs of control animals show, as in spring, diplotenic oocytes at different stages of the II growth period and several degenerating oocytes. On the contrary, the Bidder's organs of DSD treated animals are

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