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MATURATION OF POSITIVE FEEDBACK ACTION OF ESTRADIOL AND ITS INHIBITION BY PROLACTIN IN FEMALE RATS

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SUMMARY

In recent publications it has been shown that serum estradiol and FSH levels are very high in female rats between day 10 and 20 after birth and that at this time more or less regular « preovulatory type » LH peaks occur. These LH peaks could be prevented completely by administration of estrogen-antiserum (E-AS) suggesting a positive feedback action of estradiol on pituitary LH release. E-AS treatment on the other hand further increased serum FSH levels which indicates that physiologically active estradiol levels were too low to keep the FSH levels low also, hence a « castration » type effect occurred for the FSH regulatory mechanisms. At the time when estradiol and FSH levels decrease and the LH peaks do not occur anymore serum prolactin and progesterone levels steadily increase until puberty. Evidence is given in the present contribution that hyperprolactinemia induced either by injections of prolaction or by small lesions in the median eminence or by pituitary transplants under the kidney capsule result in precocious puberty. Conversely, adrenalectomy at day 23 which results in low prolactin levels delays puberty and progesterone treatment which reportedly also reduces prolactin levels delays puberty also. Both effects potentiate in adrenalectomized and progesterone treated animals. In view of data in the literature that reduction of serum prolactin levels by the use of 2-Br-ergocryptine, a dopamine receptor stimulating drug, does not delay puberty it is suggested that the timing effect of prolactin on the onset of puberty is mediated by the stimulating properties of prolactin on dopamine turnover.

The prevailing theory about gonadotropin control mechanisms in immature female rats was put forward by RAMIREZ and MCCANN (1963). These, and later the same (MCCANN and RAMIREZ, 1964) and other authors (DAVIDSON, 1969 ; ELDRIDGE *et al.*, 1974) postulated that estrogens in immature female rats meet a highly sensitive negative feedback system which suppresses pituitary LH and FSH release.

During puberty the highly estrogen sensitive « gonadostat » becomes less sensitive, hence serum gonadotropin levels rise and puberty occurs. On the other hand serum estradiol levels between day 10 and 20 postnatally have been reported to be very high (WEISZ and GUNSALUS, 1973 ; MEIJS-ROELOFS *et al.*, 1973 ; DÖHLER and WUTTKE, 1975). According to the above mentioned theory these high estrogen levels should coincide with low serum LH and FSH levels. DÖHLER and WUTTKE (1974, 1975) and WUTTKE, DÖHLER and GELATO (1976) reported in detailed studies involving more than 4000 immature female rats premature « cyclical activity » between day 10 and 20 with high FSH levels and preovulatory type LH peaks. These high FSH levels decrease and LH no longer shows peak values around day 20. At this time serum prolactin and progesterone levels begin to rise until puberty (DÖHLER and WUTTKE, 1974, 1975) whereas serum LH and FSH levels remain low until the first true preovulatory gonadotropin surges which are associated with rupture of the vaginal membrane indicating increased estrogenic activity. The role of increasing prolactin and progesterone levels between day 20 and puberty remains unclear. It was suggested that prolactin may possibly exert an inhibitory action on gonadotropin release (DÖHLER and WUTTKE, 1975).

This study presents results obtained in our laboratory during the last 3 years including results which in part elucidate the role of estrogens and prolactin and which also indicate adrenal involvement in maturational processes in female rats.

MATERIAL AND METHODS

The immature female Sprague-Dawley rats were weaned in our own animal quarters. Unless otherwise stated all age groups and differentially treated rats were decapitated between 16.00 and 18.00 h.

Treatment, age and size of groups.

1. — *Treatment with estrogen-antiserum* (E-AS)

1 *a*) 31 animals were injected twice daily with 50 µl of a potent, undiluted estrogen antiserum from day 8-15. This E-AS bound 50 p. 100 of tritiated estradiol (5-6 pg) *in vitro* at a dilution of 1 : 20 000. Furthermore, it bound estrone, estradiol and estriol equally potent but did not significantly cross-react with any other steroid. Eight to ten h after injection of this E-AS the serum of the treated rats still bound 50 p. 100 of the tracer hormone at a dilution of 1 : 1 400. As the E-AS was produced in rabbits, one control group (29 animals) of this experiment received the same injection schedule with normal rabbit serum (NRS), another control group (14 animals) remained untreated. Treated and control animals were decapitated at day 15 between 16.00 and 18.00 h.

1 *b*) 25 rats 15 days of age received a single injection of E-AS at 9 am. The animals were decapitated between 16.00 and 18.00 h at the same day, as were the NRS treated (15 rats) and untreated (15 rats) controls.

2. — *Hormone treatment from day 8-25*

2 *a*) *Prolactin treatment.*

108 animals were injected with 0.5 µg/g body weight highly purified ovine prolactin (NIH-P-S11, 26.4 IU/mg) twice daily beginning at day 8 until day 25. Controls were similarly treated with saline, the carrier solution for prolactin. Subgroups of these animals were decapitated at day 15, 25 (*i.e.* the last day of prolactin treatment) and at the day of vaginal opening.

2 *b*) *Progesterone-treatment.*

13 Animals were daily injected with 2.0 µg progesterone s.c. dissolved in corn oil beginning at day 8 until day 25. Subgroups were sacrificed at day 15 and at the day of vaginal opening. The controls (18 animals) received corn oil only.

3. — *Small lesions of the median eminence*

32 animals were lesioned in the median eminence at the age of 23 days. A concentric bipolar stainless steel electrode was stereotaxically lowered into the median eminence using coordinates worked out in our laboratory. The anodal direct current applied through the electrodes was 0.5 mA for 30 sec. Subgroups of the lesioned and sham lesioned animals were decapitated 3 h, 24 h. 4 days and 12 days later.

4. — *Pituitary grafts*

60 animals were grafted with one anterior pituitary per animal under the kidney capsule at the age of 23 days. Donor rats for the pituitaries were male rats of the age of 60-80 days. Beginning with day 25 pituitary grafted and sham operated groups of rats were decapitated every 2 days until the day of vaginal opening.

5. — *2-Br-α-ergocryptin* (CB 154)*-treatment*

Subcutaneous injections of 2 × 0.6 µg CB 154/g bodyweight/day was begun at day 18 and subgroups were decapitated at day 25, 35, at the day of vaginal opening and at the next proestrous day following the day of vaginal opening.

Blood samples from all animals were collected from the trunk and the sera assayed at the end of each experiment. Radioimmunoassays for rat FSH and prolactin were done with the NIAMDD kits kindly provided by the NIAMDD Rat Pituitary Hormone Distribution Program (Dr. A. F. PARLOW). Serum LH was determined by the heterologous assay described by NISWENDER *et al.* (1969). The values of these hormones will be stated in terms of RP-1. The specification of antisera used for radioimmunoassays of progesterone and estradiol levels have been given in detail elsewhere (DÖHLER and WUTTKE, 1974, 1975).

RESULTS

1 *a*. — *Treatment with estrogen antiserum* (E-AS), *day 8-15*

Twice daily injections of 50 µl E-AS from day 8-15 reduced LH levels in most animals to undetectable levels at day 15 and only few rats had basal LH levels. In no case elevated or peak LH levels could be determined which could readily be measured in about 60-70 p. 100 of the NRS treated and untreated animals, (fig. 1, $p < 0.005$). Serum FSH levels which are very high in untreated rats of this age are even further increased after treatment with E-AS (fig. 1, $p < 0.01$).

1 *b*. — *Treatment with E-AS, day 15*

Injections of 50 µl E-AS on the morning of day 15 did not change LH and FSH levels 8-10 hrs later (fig. 2).

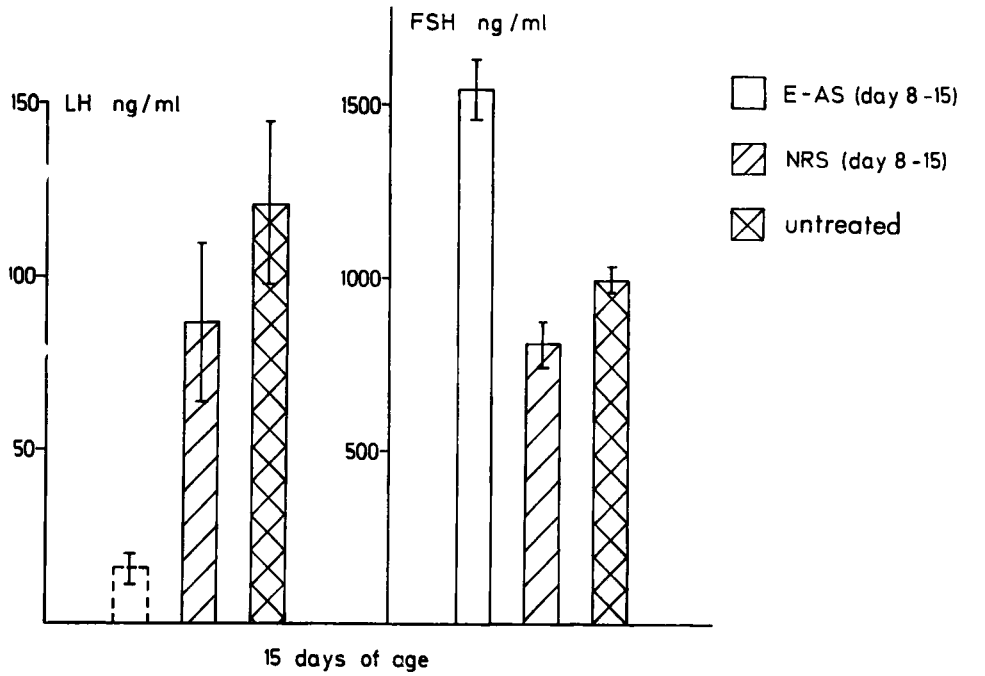

FIG. 1. — *Effect of treatment with estrogen antiserum* (E-AS) *from day 8-15 on serum LH and FSH levels.* LH levels were undetectable in most E-AS treated animals. Values shown in graph give levels in treated animals where measurable LH was present in serum. Note increased FSH levels in treated animals. Standard error of mean (SEM) on top of each bar (From WUTTKE *et al.*, 1976).

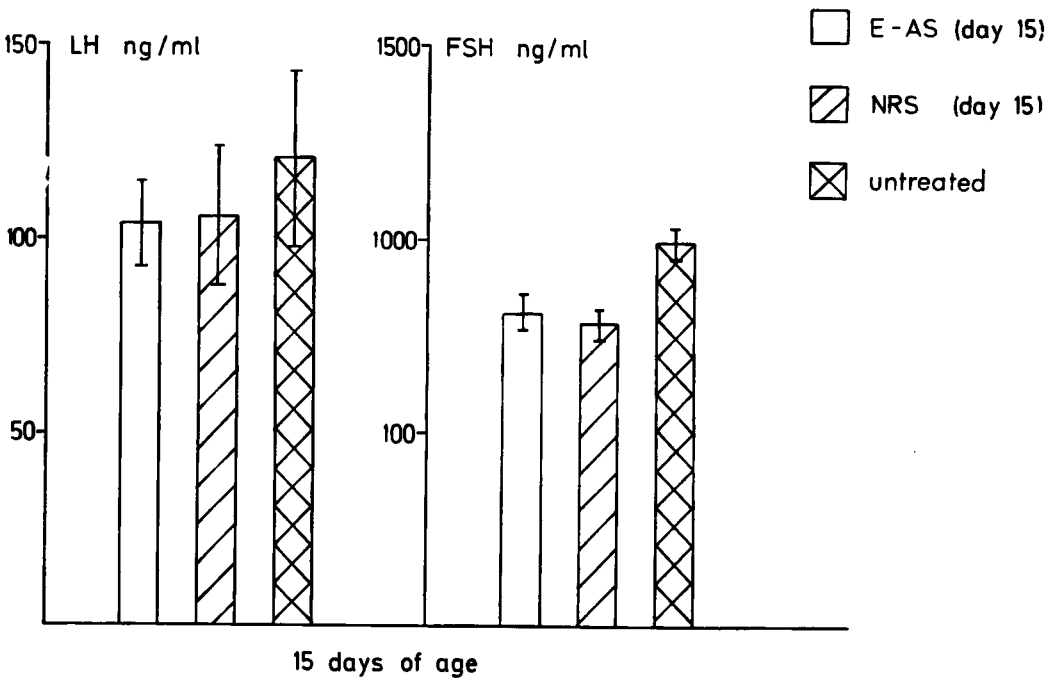

FIG. 2. — *Single injection of E-AS had no effect on serum LH and FSH levels* (SEM on top of each bar) (From WUTTKE *et al.*, 1976)

2. — Injections of prolactin or progesterone from day 8-25

2 *a*) *Injections of ovine prolactin* (2 × 0.5 µg/g BW/day) beginning at day 8 completely prevented any of the LH peaks and suppressed LH levels to values which are considered to be diestrous in adult female rats (fig. 3) whereas typical high fluctua-

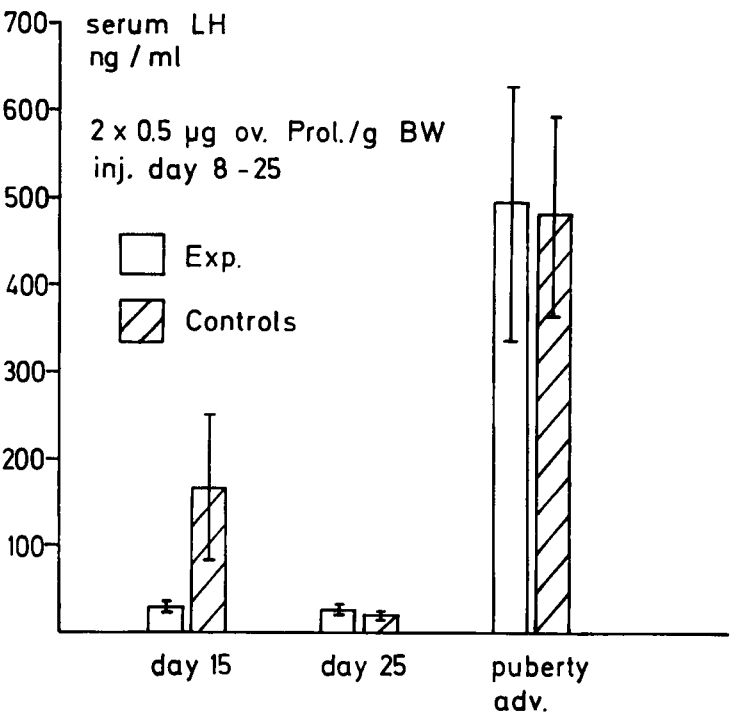

FIG. 3. — *Prevention of « preovulatory type » of LH peaks at day 15 by injection of ovine prolactin* (SEM on top of each bar)
(From WUTTKE *et al.*, 1976)

tions could be observed in the control animals. LH levels in prolactin treated animals were equally low day 25 as in the controls.

Prolactin treatment, although discontinued at day 25 significantly advanced puberty, *i.e.* the day of vaginal opening associated with the preovulatory LH surge, by a full week (34.8 ± 1.4 day *vs.* 41.3 ± 1.0 days in the controls, $p < 0.001$). Serum FSH was not changed at any age in the prolactin treated animals (e.g. day 15 650.0 ± 50.5 ng/ml compared to controls, 555.5 ± 48 ng/ml) whereas serum progesterone levels were suppressed during the treatment period (e.g. day 15 0.9 ± 0.12 ng/ml *vs.* 2.01 ± 0.3 ng/ml in controls, $p < 0.01$). Injections of ovine prolactin (which does not cross react in the homologous RIA for rat prolactin) significantly suppressed endogenous prolactin levels to basal values (27.5 ± 1.8 ng/ml) whereas the levels in control rats had increased by day 25 (80.5 ± 12.5 ng/ml, $p < 0.005$). Prolactin treatment did not increase the weights of the pups when compared with control animals of the same age. The average weight of the prolactin treated animals

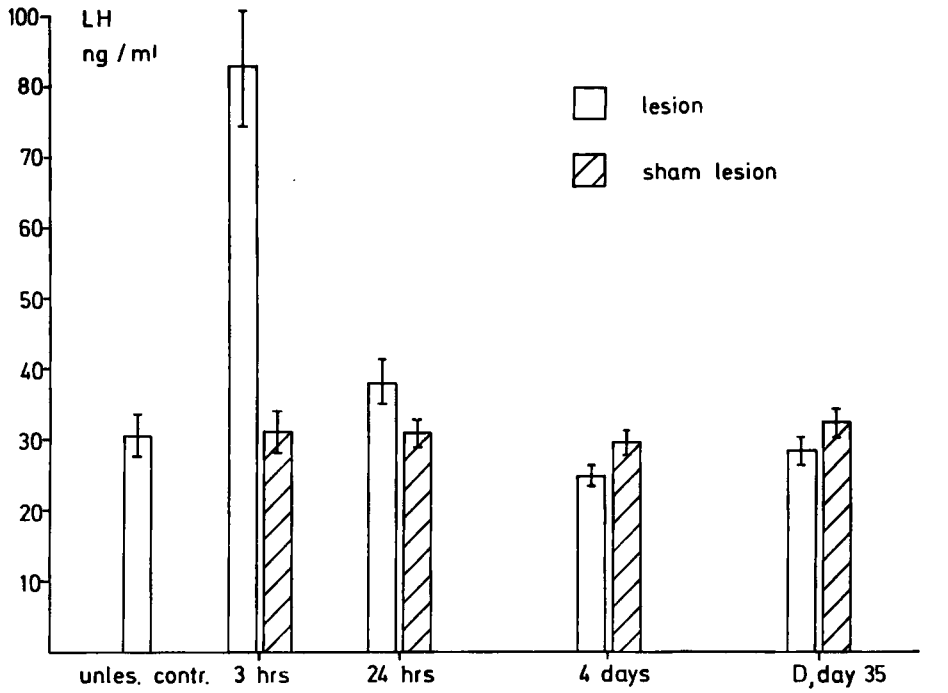

FIG. 4. — *Serum LH levels after lesions of the mediobasal hypothalamus* (MBH) (SEM on top of each bar). Day 35 or 36 was a diestrus days in lesioned animals. Controls had still closed vaginal membranes. (ENGELBART and WUTTKE, unpublished results).

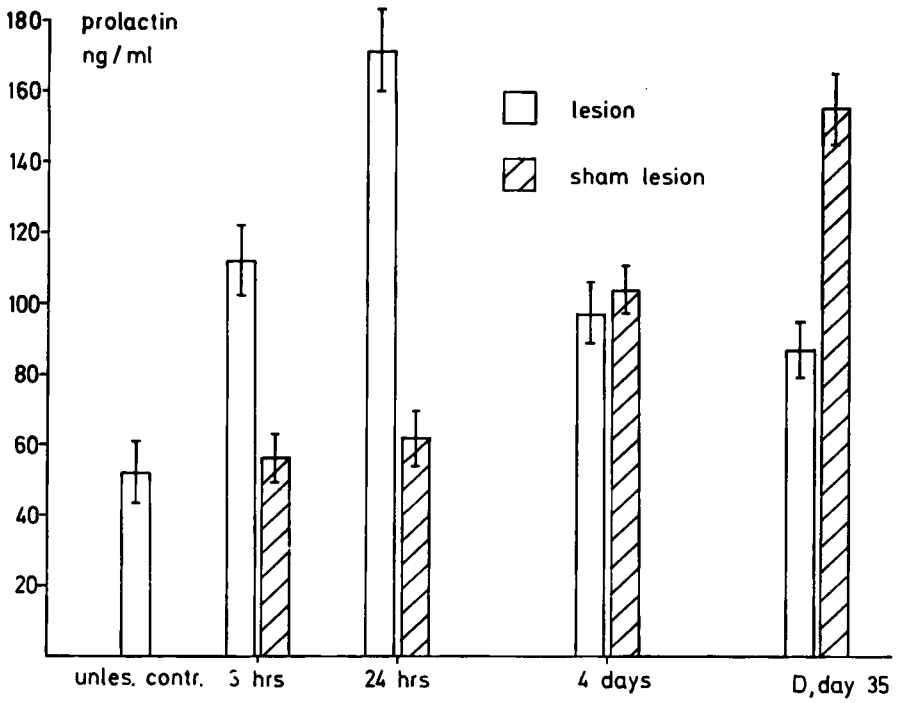

FIG. 5. — *Serum prolactin levels after MBH-lesions and in controls* (SEM on top of each bar) (ENGELBART and WUTTKE, unpublished results)

at the day of vaginal opening was 93.3 g whereas the weight at the day of vaginal opening of control rats was 112.4 g.

2 *b*) *Progesterone treatment.*

Injection of 2.0 µg progesterone/animal/day from day 8-15 did not alter either high FSH or low prolactin levels nor did it block the great variability in serum LH levels.

3. — *Small lesions of the median eminence*

Small lesions in the middle part of the median eminence of 23 day old rats resulted in precocious puberty in all animals with advancement of the day of vaginal opening to day 30.1 ± 1.2 as compared to day 39.3 ± 1.8 in sham-lesioned animals. In a subgroup which was sacrificed 3 hours after placement of the lesion serum LH (fig. 4) and prolactin (fig. 5) levels were significantly increased. One and 4 days later

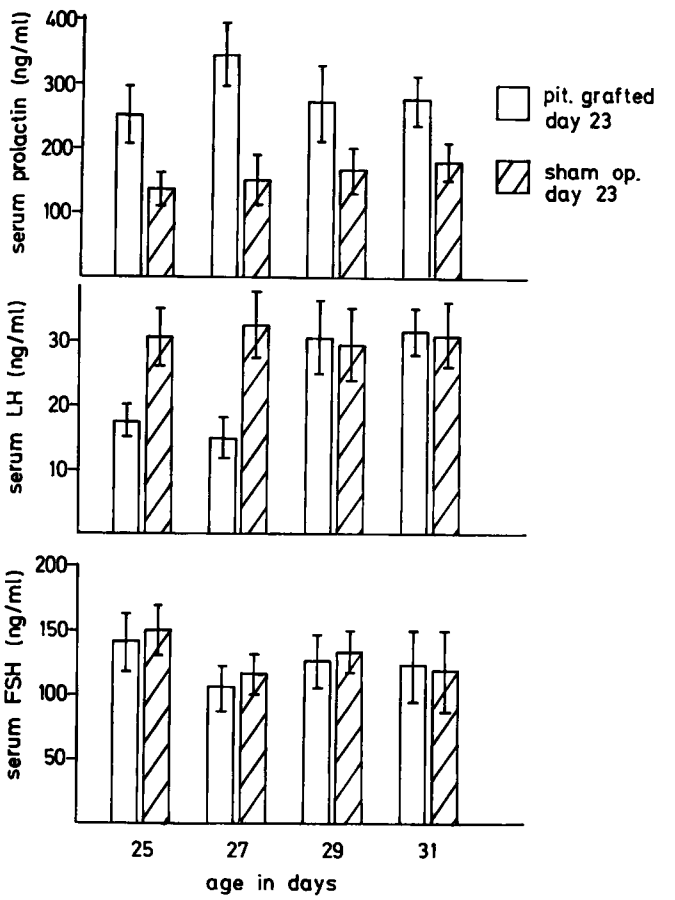

FIG. 6. — *Serum prolactin, LH and FSH levels in pituitary grafted rats.* Note higher prolactin levels and very low LH levels in pituitary grafted rats. Serum FSH levels were unchanged (SEM on top of each bar) (ENGELBART and WUTTKE, unpublished results).

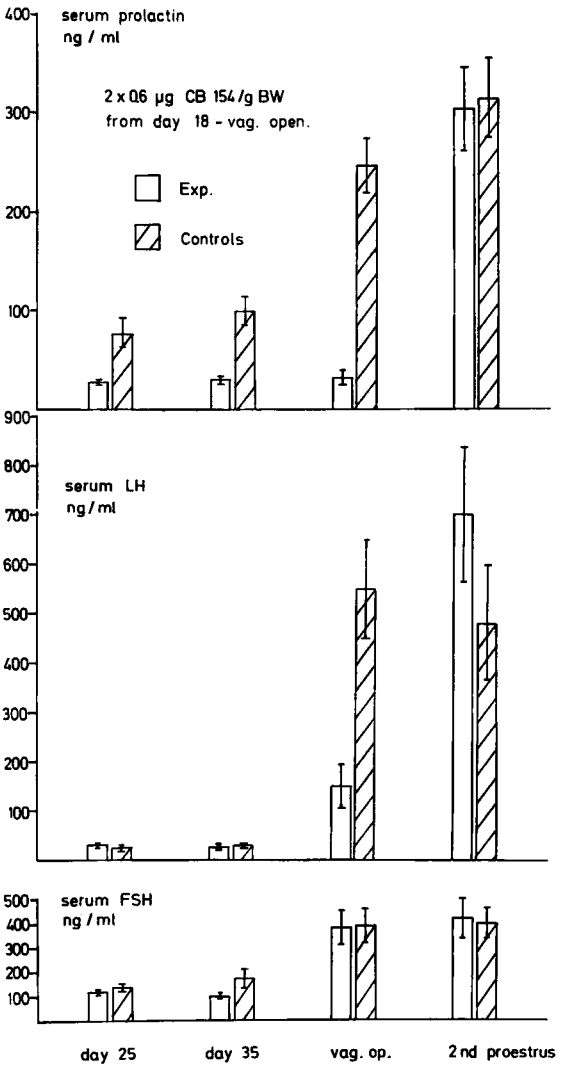

FIG. 7. — *Serum prolactin, LH and FSH levels in CB-154 treated rats.* (Treatment from day 18-vaginal opening). Note depressant action of drug on prolactin levels and first preovulatory LH peak at day of vaginal opening (SEM on top of each bar) (DÖHLER, ENGELBART and WUTTKE, unpublished results).

serum LH levels were at low control levels, serum prolactin levels however, were significantly increased above control levels by 24 hours after placement of the lesions. Four days after sham lesioning the prolactin levels in control rats had also increased and were as high as in the lesioned animals. At either day 35 or 36, which was a diestrous day in the lesioned animals prolactin levels were still higher than are considered to be normal in adult diestrous rats but significantly lower ($p < 0.01$) than in the still prepubertal controls. After puberty, the lesioned animals had rather irregular estrous cycles with prolonged diestrous periods.

4. — *Pituitary grafts*

Pituitary transplants under the kidney capsule into 23 day old rats significantly advanced the onset of puberty to 32.3 ± 1.1 days as compared to 38.2 ± 0.8 days in control animals. Serum prolactin levels in pituitary grafted rats (fig. 6) were above control levels at all times tested but were highest 2, 4 and 6 days after operation. The levels then slightly declined whereas they were rising in control rats.

Serum LH levels, which were uniformly low in sham operated controls seemed to be even lower in pituitary grafted rats but serum FSH levels were unchanged.

5. — *CB-154 treatment*

Daily injections of 2 × 0.6 µg CB 154/g bodyweight/day beginning at day 18 completely suppressed increased serum prolactin levels which are normally seen in control animals between day 20 and puberty (fig. 7). Serum LH and FSH levels remained at the low control levels. CB-154 treatment was continued until the day of vaginal opening which was not different from the day of vaginal opening in control rats (day 40 ± 1.2). Serum LH levels in these pubertal animals were lower than in control rats at vaginal opening whereas the FSH levels were in the normal range on this day. In another subgroup of animals which were killed on the next proestrous day, *i.e.* the second proestrus, 4-5 days after discontinuation of CB-154 treatment, normal preovulatory LH levels were measured.

DISCUSSION

A positive feedback action of estradiol on gonadotropin release in immature female rats has been demonstrated in animals well over 20 days of age, *i. e.* at time when under physiological conditions serum estradiol levels are very low (YING and GREEP, 1971, YING *et al.*, 1971 ; YING and GOVE, 1973). It has also been demonstrated in these older prepubertal animals that estradiol levels must be at very high levels for a certain period of time in order to stimulate pituitary gonadotropin-release (CHENG and JOHNSON, 1974). On the other hand it has been postulated by MCCANN and RAMIREZ (1964) and later also by others (DAVIDSON, 1969, ELDRIDGE *et al.*, 1974) that in the immature rat a highly sensitive negative feedback action of estradiol on gonadotropin release may be functional, keeping gonadotropin release in

check. This highly sensitive « gonadostat » was proposed to become less sensitive at puberty, hence gonadotropin release and ovulation occurred.

On the basis of radioimmunological determination of serum LH, FSH and estradiol it is now known that estradiol and FSH levels are extremely high in immature female rats at 10-20 days of age (MEIJS-ROELOFS *et al.*, 1973) whereas « preovulatory type » LH-peaks occur approximately once every 24 hours either in the afternoon or early morning hours (DÖHLER and WUTTKE, 1974, 1975). These LH peaks were proposed to be the result of high estradiol levels (DÖHLER and WUTTKE, 1974, 1975). The present data confirm this suggestion in that reduction of circulating estrogen levels by passive immunization with estrogen-antiserum from day 8-15 completely prevents the occurrence of LH surges on the afternoon of day 15 and even depresses basal LH levels to undetectable values in most animals. Serum FSH levels in the same animals are even further increased than in the NRS-treated or untreated controls which suggests the existence of a less sensitive negative feedback system for FSH than for LH in female rats of this age. Apparently E-AS treatment resulted in a castration-type effect for the FSH regulatory mechanism (s) whereas minute amounts of estrogens which were not bound by antibodies were still able to prevent a castration-type effect for pituitary LH release. By the use of the anti-estrogenic compound (I.C.I. 46474) DÖHLER *et al.* (this volume) were also able to decrease serum LH levels but this treatment also decreased FSH values. These results indicate to us that this treatment only moderately prevented the estrogens from their positive feedback action on gonadotropin release. The decreased FSH levels however, suggest that estrogens also exert a positive feedback action on FSH secretion. Thus, minute amounts of estrogens are sufficient to prevent a castration effect on LH secretion, higher levels are required for the FSH-« gonadostat » and very high levels are necessary for a positive feedback action of estrogens, resulting in both increased LH and FSH release. An alterative but less likely explanation would be that this anti-estrogenic compound may have some yet unexplored intrinsic steroidic activity. Experiments done in actively immunized adult rabbits and rats suggest that approximately 98 to 99.5 p. 100 of the steroid should be bound to the antibodies present in the injected antiserum (NIESCHLAG *et al.*, 1973, 1975). A single injection of the E-AS on the morning of day 15 did not cause any hormonal changes indicating that estrogen deprivation must take place over a longer period of time, in other words the priming effect of estrogens to induce such LH peaks on the afternoon had already occurred prior to injection of the antiserum. This LH-peak blocking effect of the E-AS and the timing of this effect are very similar to the situation in the adult rat where the preovulatory LH peak can be blocked by E-AS administration one day prior to the proestrous day but not if the E-AS is injected on the morning of proestrus (FERIN *et al.*, 1969). We therefore refer to these LH peaks which occur between day 10 and 20 as « preovulatory type » LH peaks which are similar to those evoked in the adult proestrous rat by high estrogen levels. Prolactin treatment also blocks the « preovulatory type » LH peaks without altering serum FSH levels. Continuation of such treatment until day 25 leads to precocious puberty. Similar observations were reported by others, who continuously injected prolactin until vaginal opening (CLEMENS *et al.*, 1969), and a direct effect of this hormone at the hypothalamic level was shown by implantion of prolactin into the mediobasal hypothalamus (VOOGT and MEITES, 1971). Similarly, small lesions of

the mediobasal hypothalamus result in precocious puberty (DONOVAN and VAN DER WERFF TEN BOSCH, 1956 ; BLOCH and GANONG, 1971 ; MEIJS-ROELOFS and MOLL, 1972). In this report we confirm these data and show that only a rather brief LH surge occurs due to the lesioning procedure. On the other hand serum prolactin levels are continuously high in these lesioned animals. Also pituitary transplants into otherwise intact animals results in hyperprolactinemia and precocious puberty. All these results are highly suggestive that prolactin plays an important role in the timing of the onset of puberty. The following may be a possible explanation of how prolactin can alter the day of vaginal opening. High serum prolactin levels were reported to stimulate dopamine turnover in the hypothalamus (HÖKFELT and FUXE, 1972, OLSON *et al.*, 1972). This high dopamine turnover is the cause for low endogenous prolactin levels in rats treated with ovine prolactin since dopamine has been shown to inhibit pituitary prolactin release (SHAAR and CLEMENS, 1974). Furthermore, there is good evidence that dopamine also inhibits LRF release (HÖKFELT and FUXE, 1972), hence between day 10 and 20 at times of low prolactin levels dopamine turnover should be low and estradiol may exert its positive action on LH release. At times of increasing prolactin levels, dopamine turnover is also increased which inhibits LRF release, hence the « preovulatory type » LH peaks come to an end. An additional factor preventing this premature gonadotropic acitivity may be the decreased estradiol levels.

It has been shown in a number of neurochemical and electrophysiological experiments that cholinergic as well as catecholaminergic postsynaptic membranes become hypersensitive to the appropriate neurotransmitter if deprivated of this transmitter for a longer period of time. (CANNON and ROSENBLUETH, 1949 ; MILEDI, 1960; UNGERSTEDT, 1971).

Conversely the postsynaptic membrane becomes desensitized if high amounts of the appropriate neurotransmitter are available to the receptors of the postsynaptic membrane. Hence, increasing prolactin levels progressively increase dopamine turnover which in turn progressively desensitize dopamine receptors. Dopamine receptors seem to be insensitive at around day 40 in normal animals. Thus dopamine does not inhibit LRF release and therefore the first real preovulatory LH peak and puberty occur. There is evidence, in the literature for these assumptions : it has recently been shown by MARKO and FLÜCKIGER (1974) that the potent dopamine receptor stimulating drug 2-Br-α-ergocryptin (CB-154) is 13.3 times more potent in immature rats of the age of 26 days than in adult female rats. The effective dose of this drug to prevent PMS induced ovulation in 50 p. 100 of the animals (ED_{50}) was 1.5 mg/kg in the immature rat but 20 mg/kg in the adult proestrous rat. These observations seem to confirm our assumption that the dopamine receptors are more sensitive to dopamine at day 26 than in adulthood. They furthermore offer an explanation for the blockade of pituitary prolactin release from day 18 until puberty having no effect on the onset of puberty. Low prolactin levels during the prepubertal time should alter the onset of puberty however, CB-154 mimicks the effect of prolactin, thus counteracting the prolactin deprevation effect.

Finally, the results presented by GELATO *et al.* (this volume) indicate that adrenalectomy at day 21 delays the onset of puberty. This observation is in agreement with findings-reported by GORSKI and LAWTON (1973), RAMALEY and BARTOSIK (1975). Our results further demonstrate that adrenalectomy results later in less

pronounced increases in serum prolactin levels. This effect of adrenalectomy can be counteracted by treatment with prolactin. Thus, it seems that the lower prolactin levels in adrenalectomized immature rats is the cause for delayed puberty. Progesterone has been shown to depress pituitary prolactin release in adult rats (CHEN and MEITES, 1970). This depressant effect of progesterone on prolactin release may also be the cause for delayed puberty in progesterone treated and the further delay in adrenalectomized and progesterone treated immature rats. These observations add further evidence to the importance of prolactin as a timing factor for the onset of puberty in female rats.

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RÉSUMÉ

MATURATION DE LA RÉTROACTION POSITIVE DE L'ESTRADIOL ET SON INHIBITION PAR LA PROLACTINE CHEZ LA RATTE

Entre 10 et 20 jours après la naissance, la Ratte présente des taux très élevés de FSH et d'estradiol dans le plasma. Au même moment des décharges de LH de type « préovulatoire » se produisent plus ou moins régulièrement. Ces décharges de LH peuvent être totalement inhibées par injection d'un sérum anti-estrogène (E-AS) ce qui suggère que l'estradiol exerce une rétroaction positive sur la sécrétion de LH par l'hypophyse.

De plus, le traitement E-AS provoque une augmentation supplémentaire du taux de FSH sérique, ce qui indique que les taux d'estradiol circulant physiologiquement actifs, sont trop faibles pour maintenir la FSH à un bas niveau et qu'un effet de type « castration » intervient dans les mécanismes de régulation de la FSH.

Quand les taux d'estradiol et de FSH diminuent et que les décharges de LH ne se produisent plus, les taux de prolactine et de progestérone augmentent régulièrement jusqu'à la puberté. Si on provoque une hyperprolactinémie par injection de prolactine, lésion de l'éminence médiane, ou mise en place de transplants d'hypophyse sous la capsule rénale, on observe une puberté précoce. Inversement, la surrénalectomie à 23 jours ou un traitement par la progestérone qui maintiennent la prolactine à un bas niveau, retardent l'apparition de la puberté. Par contre, la réduction du taux de prolactine sérique par le 2-Br-ergocryptine, drogue stimulant les récepteurs dopaminergiques, ne retarde pas la puberté. Ceci suggère que l'effet de la prolactine sur l'apparition de la puberté passe par l'effet stimulant de la prolactine sur le turnover de la dopamine.

REFERENCES

BLOCH G. J., GANONG W. F., 1971. Lesions of the brain and onset of puberty in the female rat. *Endocrinology*, **89**, 898-901.

CANNON W. B., ROSENBLUETH A., 1949. *The supersensitivity of denervated structures*. Macmillan Co., New York.

CHEN C. L., MEITES J., 1970. Effects of estrogen and progesterone on serum and pituitary prolactin levels in ovariectomized rats. *Endocrinology*, **86**, 503-505.

CHENG H. C., JOHNSON D.C., 1974. Temporal changes in serum estradiol and prolactin in immature female rats given a single injection of estradiol benzoate. *Steroids*, **24**, 657-664.

CLEMENS J. A., MINAGUCHI H., STOREY R., VOOGT J. L., MEITES J., 1969. Induction of precocious puberty in female rats by prolactin. *Neuroendocrinology*, **4**, 150-156.

DAVIDSON J. M., 1969. Feedback control of gonadotropin secretion. 343-388, in : GANONG W. F., MARTINI L., *Frontiers in Neuroendocrinology*. Oxford University Press, New York,

DÖHLER K. D., WUTTKE W., 1974. Serum LH, FSH, prolactin and progesterone from birth to puberty in female and male rats. *Endocrinology*, **94**, 1003-1008.

DÖHLER K. D., WUTTKE W., 1975. Changes with age in levels of serum gonadotropins, prolactin and gonadal steroids in prepubertal male and female rats. *Endocrinology*, **97**, 898.

DONOVAN B. T., VAN DER WERFF TEN BOSCH J. J., 1956. Precocious puberty in rats with hypothalamic lesions. *Nature*, **178**, 745.

ELDRIDGE J. C., MCPHERSON J. C., MAHESH V. B., 1974. Maturation of negative feedback control of gonadotropin secretion in the female rat. *Endocrinology*, **94**, 1536-1540.

FERIN M., TEMPONE A., ZIMMERING P. E., VAN DE WIELE R. L., 1969. Effect of antibodies to 17-βestradiol and progesterone on the estrous cycle of the rat. *Endocrinology*, **85**, 1070-1078.

GORSKI M. E., LAWTON L. E., 1973. Adrenal involvement in determining the time of onset of puberty in the rat. *Endocrinology*, **93**, 1232-1234.

HÖKFELT T., FUXE K., 1972. Effects of prolactin and ergot alkaloids on the tuberoinfundibular dopamine (DA) neurons. *Neuroendocrinology*, **9**, 100-122.

HÖKFELT T., FUXE K., 1972. On the morphology and the neuroendocrine role of the hypothalamic catecholamine neurons, 181-223, in : KNIGGE K., SCOTT D., WEINDL A., *Brain-endocrine interactions. Median eminence : Structure and function*. S. Karger, Basel.

MARKO M., FLÜCKIGER E., 1974. Inhibition of spontaneous and induced ovulation in rats by non-steroidal agents. *Experientia*, **30**, 1174-1176.

MCCANN S. M., RAMIREZ D. V., 1964. The neuroendocrine regulation of hypophyseal luteinizing hormone secretion. *Rec. Progr. Horm. Res.*, **20**, 131-170.

MEIJS-ROELOFS H. M. A., UILENBROEK J. Th. J., DE JONG F. H., WELSCHEN R., 1973. Plasma oestradiol-17β and its relationship to serum follicle-stimulating hormone in immature female rats. *J. Endocr.*, **59**, 295-304.

MEIJS-ROELOFS H. M. A., MOLL J., 1972. Differential effects of anterior and middle hypothalamic lesions on vaginal opening and cyclicity. *Neuroendocrinology*, **9**, 297-303.

MILEDI R., 1960. The acetycholine sensitivity of frog muscle fibres after complete or partial denervation. *J. Physiol.*, **151**, 1-23.

NIESCHLAG E., USADEL K. H., KLEY H. K., SCHWEDES U., SCHÖFFLING K., KRÜSKEMPER H. L., 1973. Alterations in testicular morphology and function in rabbits following active immunization with testosterone. *Endocrinology*, **92**, 1142-1147.

NIESCHLAG E., USADEL K. H., WICKINGS E. J., KLEY H. K., WUTTKE W., 1975. Effect of active immunization with steroids on endocrine and reproductive functions in male animals. 155-170, in : NIESCHLAG E., *Immunization with hormones in reproduction research*. North Holl and/American Elseview, Amsterdam, New York.

NISWENDER G. D., MIDGLEY A. R., MONROE S. E., REICHERT L. E., 1968. Radioimmunoassay for rat luteinizing hormone with antiovine LH serum and ovine LH-^{131}I. *Proc. Soc. Exp. Biol. Med.*, **128**, 807-811.

OLSON L., FUXE K., KÖKFELT T., 1972. The effect of pituitary transplants on the tubero-infundibular dopamine neurons in various endocrine states. *Acta endocr.*, **71**, 233-244.

RAMALEY J. A., BARTOSIK D., 1975. Precocious puberty : The effect of adrenalectomy on PMS-induced ovulation and progesterone secretion. *Endocrinology*, **96**, 269-274.

RAMIREZ D. V., MCCANN S. M., 1963. Comparison of the regulation of luteinizing hormone (LH) secretion in immature and adult rats. *Endocrinology*, **72**, 452-474.

SHAAR C. J., CLEMENS J. A., 1974. Role of catecholamines in release of anterior-pituitary prolactin *in vitro*. *Endocrinology*, **95**, 1202-1212.

UNGERSTEDT U., 1971. Histochemical studies on the effect of intracerebral and intraventricular injections of 6-hydroxydopamine on monoamine neurons in the rat brain. 101-127, in : MALMFORS T., THOENEN H., *6-hydroxydopamine and catecholamine neurons*. North Holland Publishing Co., Amsterdam.

VOOGT J. L., MEITES J., 1971. Effects of an implant of prolactin in median eminence of pseudopregnant rats on serum and pituitary LH, FSH and prolactin. *Endocrinology*, **88**, 286-292.

WEISZ J., GUNSALUS P., 1973. Estrogen levels in immature female rats : true or spurious-ovarian or adrenal ? *Endocrinology*, **93**, 1057-1065.

WUTTKE W., DÖHLER K. D., GELATO M., 1976. Oestrogens and prolactin as possible regulators of puberty. *J. Endocr.*, (*in press*).

YING S.-Y., GREEP R. O., 1971. Effect of estradiol on ovulation in immature rats primed with different doses of pregnant mare's serum (PMS). *Endocrinology*, **89**, 213-218.

YING S.-Y., FANG V. S., GREEP R. O., 1971. Estradiol benzoate (EB)-induced changes in serum-luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in immature female rats. *Fertil. Steril.*, **22**, 799-801.

YING S.-Y., GOVE S., 1973. Compensatory ovulation after unilateral ovariectomy in immature rats primed with gonadotropins. *Proc. Soc. Exp. Biol. Med.*, **144**, 22-25.