

were, however, encountered. The present study supports the results obtained by the fluorescent histochemical studies^{1,2}. It appears that only the augmentation of the NA content is fast during the critical period. Thus, the appearance of the bright green fluorescence in the median eminence might be due to the rapid increase of the NA content. On the other hand, it has been shown that the green fluorescence inside the pineal gland is visible already in the 2-day-old rat¹⁶. In the present study DA was not possible to measure at that age, and thus the early fluorescence can be due to NA in the pineal nerves.

Even though the role of DA in the adult hypothalamus for the regulation of the gonadotrophin secretion has been emphasized^{3,5}, its concentration in the early life does not seem to be related to the sexual differentiation in rat. Recently it has been supposed that the 5-HT content might be related to the process of sexual differentiation of the rat brain⁹. Its concentration in the whole brain was also modified by androgenization procedures. This observation could not be confirmed in the separate hypothalamus, though the 5-HT content in the cerebral cortex was significantly higher in female than in male rats at the age of 60 days¹⁷.

Because the 10 first days are critical for the general function of the brain¹⁸, it should not be surprising if the

content of monoamines increases. However, more detailed studies on the metabolism of the hypothalamic monoamines may confirm whether the monoamines are necessary for the sexual differentiation of the structures that control gonadotrophin secretion¹⁹.

Zusammenfassung. Der Gehalt an Monoaminen im Hypothalamus der Ratte wurde während der sexuellen Differenzierung bestimmt, wobei für das hypothalamische Noradrenalin eine signifikante Zunahme zwischen dem 4. und 10. Tag gefunden wurde.

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Feedback Effect of Estrogen on FSH Secretion

Intrahypothalamic implants of small quantities of estrogen have been shown to suppress the synthesis as well as the release of luteinizing hormone (LH)¹⁻⁴. Since brain implants of estrogen can also induce gonadal atrophy and can block ovarian compensatory hypertrophy, it has been argued that they may also inhibit follicle-stimulating hormone (FSH) secretion⁵⁻¹². However, clear-cut information based on direct measurements of FSH is still lacking. This is mainly due to the fact that the bioassay used for measuring FSH is less sensitive than that used for evaluating LH, so that reliable measurements of plasma FSH cannot be performed. This situation will undoubtedly improve soon with the development of specific and sensitive radioimmunoassays for the measurement of plasma FSH levels in animal species.

An attempt to overcome this methodological gap has been made in the experiments here to be described, by selecting appropriate experimental conditions. Minute amounts of estradiol have been placed in the median eminence of the hypothalamus or in the anterior pituitary of sexually mature male rats. Male animals have been selected because they permit us to obtain a satisfactory, even if indirect, evaluation of blood levels of FSH and LH; as is known, the weight of the testes is directly related to the amounts of FSH present in the circulation, and the weights of ventral prostates and of seminal vesicles provide satisfactory indications of the amounts of circulating LH-ICSH (interstitial cells-stimulating hormone)¹³.

Materials and methods. Adult male rats of the Sprague-Dawley strain were used in the experiments here to be described. They were allowed a standard rat pellet diet and water ad libitum. Cannulae bearing estradiol 17 β were implanted into the median eminence (ME) or the anterior pituitary gland (PIT), using a Stoelting stereotaxic instrument and KRIEG's¹⁴ atlas. Implants were

fixed to the skull with dental cement and left in situ for 5 days.

At the end of the experiment, animals were sacrificed using a guillotine. The fresh weights of the pituitaries, testes, prostates and seminal vesicles were recorded. Because of the observation¹⁵⁻¹⁷ that male rats exhibit a diurnal variation in the concentrations of gonadotropins in their pituitaries, all animals were killed at the

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Table I. Effect of implants of estradiol in the median eminence (ME) and in the anterior pituitary (PIT) on organ weights of male rats^a

Groups ^b	Body weight (g)	Pituitary weight (mg)	Testes weight (g)	Prostates weight (mg)	Seminal vesicles weight (mg)
ME: sham (60)	231 ± 4.1	11.0 ± 0.39	2.71 ± 0.53	182.9 ± 7.8	288.8 ± 16.0
ME: estradiol (60)	233 ± 2.6	13.1 ± 0.45 ^c	2.53 ± 0.43 ^c	129.1 ± 7.5 ^d	151.8 ± 9.2 ^d
PIT: sham (20)	265 ± 11.0	8.9 ± 0.40	2.97 ± 0.70	179.8 ± 13.0	404.0 ± 17.5
PIT: estradiol (20)	278 ± 6.0	12.1 ± 0.60 ^c	2.80 ± 0.50	162.2 ± 12.5	346.0 ± 14.8 ^c

^a Values are means ± S.E. ^b Number of rats in parentheses. ^c $P < 0.01$. ^d $P < 0.001$.

Table II. Effect of implants of estradiol in the median eminence (ME) and in the anterior pituitary (PIT) on pituitary FSH concentrations of male rats

Groups ^a	Pituitary FSH (µg/mg) ^b	Limits (95%)
ME: sham (2)	44.83 54.31	(38.7–54.1) (47.2–62.1)
ME: estradiol (2)	26.73 33.33	(19.1–32.3) (27.2–39.5)
PIT: sham (2)	40.40 36.32	(30.2–48.3) (25.4–40.5)
PIT: estradiol (2)	32.82 28.73	(22.8–39.4) (20.3–32.4)

^a Number of assays in parentheses. ^b Microgram equivalents of NIH-FSH-S-3 ovine per mg wet weight of pituitary.

same hour of the day, i.e., between 16.00 and 17.00 h; at this time the concentration of gonadotropins in the pituitary is particularly elevated.

The concentration of FSH in the pituitaries of the different groups of animals was measured in homogenates of pooled glands by using the ovarian augmentation test of STEELMAN and POHLEY¹⁸. A 2 + 2 design was adopted against a standard of FSH (NIH-FSH-S-3 ovine). Five assay animals were used for each point.

Results and discussion. The implantation of estradiol into the median eminence results in a significant decrease in the weights of the testes, of the prostates, and of the seminal vesicles (Table I). This suggests that the amounts of FSH and of LH in the general circulation are significantly diminished by the presence of estradiol in this region of the brain. Intrapituitary implants have been completely ineffective on testicular weights, although they have induced a moderate decrease of the weights of the prostates and of the seminal vesicles; only the decrease of the weight of the seminal vesicles is significant (Table I). It appears that intrapituitary estrogen does not modify the release of FSH but probably brings about some reduction of the release of LH; this confirms previous data⁴.

Median eminence implants of estradiol have been shown to induce a significant reduction of the concen-

tration of FSH in the pituitary (Table II); the 'total' content of FSH per pituitary is also reduced, in spite of the increased pituitary weight found in animals bearing hypothalamic implants of estradiol. Intrapituitary implants caused a small, statistically insignificant reduction in the concentration of FSH; however, because of the increase in the pituitary weight found in this group of animals, the total amount of FSH per pituitary is enhanced.

The presence of atrophy of the testes indicates that the decreased pituitary levels of FSH observed following median eminence implants of estrogen cannot be ascribed to a stimulation of the release of the hormone; the data suggest, then, that estrogen, when present in high concentration on receptors located in the median eminence region, can reduce the synthesis as well as the release of FSH.

The data here reported provide the first direct demonstration of the existence in the hypothalamus of estrogen receptors involved in the control of FSH secretion; apparently estrogen receptors for the feedback control of FSH are not present at pituitary level¹⁹.

Résumé. L'implantation de petites quantités d'œstradiol dans l'éminence médiane de l'hypothalamus de rats mâles est suivie par l'atrophie des testicules, des prostates et des vésicules séminales. Au niveau de l'hypophyse des animaux implantés, on rencontre une diminution très significative des concentrations de FSH. Les résultats démontrent d'une façon directe que l'éminence médiane contient des récepteurs sensibles aux œstrogènes, et qui interviennent dans le contrôle de la sécrétion de la FSH.

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