Hypothalamic Monoamines and Pineal Dopamine During the Sexual Differentiation of the Rat Brain

It has recently been shown with a histochemical method that the primary catecholamines in the median eminence of the hypothalamus appear for the first time at the 5th postnatal day of the rat[1,2]. Some fluorescent nerve fibers have been observed also before birth[1]. On the other hand, it is wellknown that the 10 first postnatal days are critical for the sexual differentiation of the rat brain[3–5]. In the adult rat hypothalamic monoamines are necessary for the regulation of gonadotrophin secretion[6–8]. Some results indicate that the concentration of 5-hydroxytryptamin in the brain may be related to the sexual differentiation[9]. However, it is not clear whether the hypothalamic monoamines (dopamine, DA; noradrenaline, NA; 5-hydroxytryptamin, 5-HT) are related to the process of the sexual differentiation of the brain.

The aim of the present study is to clarify whether there are sudden changes in the content of the hypothalamic monoamines during the critical period of sexual differentiation. The DA content in the pineal gland during the postnatal days was also analyzed.

Material and method. The material consists of 326 albino rats. In the groups of the youngest rats (0–1-, 4-, 10-day-old) both sexes were studied separately, whereas only male rats were used in the older age-groups (30, 60 days). Rats were kept in a heat-stable (+25 °C), humidity-controlled (60%) room with constant light period (08.00–20.00 h). The animals were killed between 09.00–11.00 h. NA and 5-HT were analyzed microchemically from the hypothalamus and cerebral cortex according to MAICKEL et al.[10]. DA was determined from the hypothalamus, forebrain or cerebral cortex, and pineal gland by the method of LAVERTY and SHARMAN[11]. Each sample consisted of the tissue extract taken from 3–6 individual animals. The pineal glands were pooled from 10–15 animals to make one sample.

Results and discussion. The content of NA increased highly significantly from the 4th to the 10th day (Table I). Its content was quite low at birth but increased approximately threefold until the 60th day, when it approached the adult level. The content of DA (Table II) and 5-HT was low at birth and increased slowly and gradually without any fast steps. No sexual differences were seen in the concentration of any monoamine in 0–1-, 4- and 10-day-old rats. In the pineal gland the content of DA was not measurable before 10 days of age, when it was approximately 4 ng/gland (Table II).

Previously the content of DA, NA, and 5-HT have been quantified in the whole rat brain[9,12,13] and even in some parts of the brain[14] during the ontogenic development. However, the concentrations of the monoamines in the hypothalamus which is important for the sexual differentiation have not been measured. Only KRAGT et al.[15] have analyzed hypothalamic DA and NA quantitatively during the puberty of the rat. No clear changes

[1] M. HYYPPÄ, Z. Zellforsch. *98*, 550 (1969).
[2] G. C. SMITH and R. W. SIMPSON, Z. Zellforsch. *104*, 541 (1970).
[3] K. FUXE and T. HÖKFELT, in *Neurosecretion* (Ed. F. STUTINSKY; Springer, Berlin, Heidelberg, New York 1967), p. 165.
[4] J. A. COPPOLA, J. Reprod. Fert., Suppl. *4*, 35 (1968).
[5] I. A. KAMBERI, H. P. G. SCHNEIDER and S. M. MCCANN, Endocrinology *86*, 278 (1970).
[6] G. W. HARRIS, Endocrinology *75*, 627 (1964).
[7] R. A. GORSKI, J. Reprod. Fert., Suppl. *1*, 67 (1966).
[8] J. SZENTAGOTHAI, B. FLERKÓ, B. MESS and B. HALASZ, *Hypothalamic Control of the Anterior Pituitary* (Akadémiai Kiadó, Budapest 1968).
[9] W. LADOSKY and L. C. J. GAZIRI, Neuroendocrinology *6*, 168 (1970).
[10] R. P. MAICKEL, R. H. COX JR., J. SAILLANT and F. P. MILLER, Int. J. Neuropharmac. *7*, 275 (1968).
[11] R. LAVERTY and D. F. SHARMAN, Br. J. Pharmac. *24*, 538 (1965).
[12] H. C. AGRAWAL, S. N. GLISSON and W. A. HIMWICH, Biochem. biophys. Acta *130*, 511 (1966).
[13] P. C. BAKER and W. B. QUAY, Brain Res., Amsterdam *12*, 273 (1969).
[14] L. A. LOIZOU and P. SALT, Brain Res., Amsterdam *20*, 467 (1970).
[15] C. L. KRAGT and W. F. GANONG, The Endocrine Society, Program of the Fifty-First Meeting Philadelphia 1969, p. 81.

Table I. Hypothalamic NA and 5-HT during the postnatal development of the rat

Days	0	4	10	30	60
NA					
Hypothalamus	0.42 ± 0.18 (7)	0.49 ± 0.10 (7)[a]	0.81 ± 0.20 (9)[b]	1.25 ± 0.49 (9)	1.35 ± 0.32 (13)
Cerebral cortex	0.20 ± 0.04 (7)	0.18 ± 0.11 (7)	0.25 ± 0.04 (9)[c]	0.46 ± 0.08 (7)	0.58 ± 0.21 (11)
5-HT					
Hypothalamus	0.54 ± 0.04 (3)	0.67 ± 0.02 (5)	0.69 ± 0.17 (5)[b]	1.19 ± 0.43 (11)	1.07 ± 0.37 (14)
Cerebral cortex	0.45 ± 0.24 (3)	0.35 ± 0.02 (5)	0.48 ± 0.14 (5)	0.53 ± 0.14 (9)	0.60 ± 0.20 (13)

Table II. Hypothalamic and pineal DA during the postnatal development of the rat

Days	1	4	10	30	60
Hypothalamus	0.20 ± 0.07 (5)	0–0.19 (4)	0.17 ± 0.04 (5)	0.21 ± 0.09 (3)	0.24 ± 0.11 (4)
Forebrain	0.24 ± 0.08 (5)	0–0.07 (4)	—	—	—
Cerebral cortex	—	—	0.05 ± 0.01 (4)	0.08 ± 0.04 (4)	0.19 ± 0.11 (4)
Pineal (ng/gland) gland	0	0	4	10	12

Means μg/g ± S.D. In parenthesis the number of extracts obtained from 3–6 individual rats. [a] Highly significant difference between the 4th and 10th day. [b] Almost significant and [c] highly significant difference between the 10th and 30th day.

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 rat[16]. 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 brain[9]. Its concentration in the whole brain was also modified by androganization 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 days[17].

Because the 10 first days are critical for the general function of the brain[18], 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 secretion[19].

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.

M. HYYPPÄ

Department of Anatomy, University of Turku, 20520 Turku 52 (Finland), 21 September 1970.

[16] C. R. S. MACHADO, L. E. WRAGG and A. B. M. MACHADO, Brain Res., Amsterdam *8*, 310 (1968).
[17] M. HYYPPÄ and U. K. RINNE, Acta endocrin., Copenh., in press (1971).
[18] W. A. HIMWICH, Int. Rev. Neurobiol. *4*, 117 (1962).
[19] Supported by grants from The Yrjö Jahnsson Foundation and the National Research Council for Medical Sciences, Finland.

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) secretion[5-12]. 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 undoubtly 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 17β were implanted into the median eminence (ME) or the anterior pituitary gland (PIT), using a Stoelting stereotaxic instrument and KRIEG's[14] 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 observation[15-17] that male rats exhibit a diurnal variation in the concentrations of gonadotropins in their pituitaries, all animals were killed at the

[1] S. KANEMATSU and C. H. SAWYER, Am. J. Physiol. *205*, 1073 (1963).
[2] S. KANEMATSU and C. H. SAWYER, Endocrinology *75*, 579 (1964).
[3] S. M. MCCANN and V. D. RAMIREZ, Recent Prog. Horm. Res. *20*, 131 (1964).
[4] V. D. RAMIREZ, R. M. ABRAMS and S. M. MCCANN, Endocrinology *75*, 243 (1964).
[5] I. CHOWERS and S. M. MCCANN, Proc. Soc. exp. Biol. Med. *124*, 260 (1967).
[6] J. M. DAVIDSON and C. H. SAWYER, Acta endocr., Copenh. *37*, 385 (1961).
[7] J. M. DAVIDSON, in *Frontiers in Neuroendocrinology* (Eds. W. F. GANONG and L. MARTINI; Oxford University Press, New York 1969), p. 343.
[8] K. FENDLER and E. ENDRÖCZI, Neuroendocrinology *1*, 129 (1966).
[9] B. FLERKÓ and J. SZENTÁGOTHAI, Acta endocr., Copenh. *26*, 121 (1957).
[10] R. D. LISK, J. exp. Zool. *145*, 197 (1960).
[11] R. D. LISK, Acta endocr., Copenh. *48*, 209 (1965).
[12] R. D. LISK, Neuroendocrinology *4*, 368 (1969).
[13] J. M. DAVIDSON, in *Neuroendocrinology* (Eds. L. MARTINI and W. F. GANONG; Academic Press, New York 1966), vol. 1, p. 565.
[14] W. J. KRIEG, Q. Bull. NWest. Univ. med. Sch. *20*, 199 (1946).
[15] F. FRASCHINI and M. MOTTA, Program 49th Meet. Endocrine Soc., p. 128 (1967).
[16] F. FRASCHINI, in *Progress in Endocrinology* (Ed. C. GUAL; Excerpta Medica, Amsterdam 1969), p. 637.
[17] L. MARTINI, F. FRASCHINI and M. MOTTA, Recent Prog. Horm. Res. *24*, 439 (1968).