Is Feminine Differentiation of the Brain Hormonally Determined?

B. H. SHAPIRO, A. S. GOLDMAN[1], H. F. STEINBECK[2] and F. NEUMANN[2]

Division of Experimental Pathology, The Children's Hospital of Philadelphia and Department of Pediatrics, University of Pennsylvania School of Medicine, 34th Street and Civic Center Boulevard, Philadelphia (Pennsylvania 19104, USA); and Department of Endocrinopharmacology, Laboratories Schering AG., Berlin (German Federal Republic, BRD), 8 December 1975.

Summary. The androgen insensitive, genetically male rat pseudohermaphrodite displays neither masculine or feminine sexual behavior when primed with the appropriate sex hormones. Although in the absence of androgen imprinting the animal develops anatomically as female, our results suggest that feminine differentiation of the brain requires active imprinting by perinatal hormone(s), possibly adrenal progesterone.

It has been generally concluded that the inherent program of sexual differentiation in both sexes of mammals is female. If androgens are present during sexual differentiation then both genetic males and females will be organized for masculine reproductive organs[3], hepatic steroidogenic enzymes[4], hypothalamic control of gonadotrophin secretion (tonic)[5] and sexual behavior[6] while absence of either gonad during the critical developmental period allows for the expression of the inborn female program[3–8]. However, our recent findings using prenatal gonadotrophic and steroidogenic blockers suggest that feminine sexual differentiation is not a passive readout of an inborn program but requires fetal pituitary and/or adrenal hormones as feminine organizing agents[9].

The genetically male rat pseudohermaphrodite's androgen insensitivity is due to a deficiency in target organ androgen binding proteins[10] which prevents masculine anatomic[11] and hepatic differentiation[12] and results in a feminine phenotype. However, we have recently reported that gonadotropin secretion in the pseudohermaphrodite is not cyclic[13,14] indicating that in the absence of androgen imprinting this parameter of sexual differentiation does not result in the expression of the female phenotype. From this observation we decided to determine whether another aspect of neural differentiation, sexual behavior, is expressed as female in the pseudohermaphrodite.

Materials and methods. Pseudohermaphrodites (8) and their normal King-X Holtzman littermate males (8) and non-littermate females (8) (Introgen, Oklahoma City, Oklahoma, USA) were housed in temperature and humidity controlled animal quarters. The illumination schedule consisted of 12 h of light and 12 h of darkness and testing commenced at the beginning of the dark period. All the rats were gonadectomized 1 month before behavioral testing.

Before testing for feminine sexual behavior rats received 0.2 mg estradiol benzoate (EB) 72 and 24 h prior to each test, and 1 mg of progesterone 6 h before testing. Observations were made in semicircular areas. Each rat was introduced into an observation cage with a male of proven sexual vigor and after 10 min of observation they were separated. This procedure of testing for feminine sexual behavior was repeated the following week. Repeated lordosis within the 10 min period was rated as positive for the feminine sexual response.

Before testing for masculine sexual behavior rats were injected, daily, with 0.1 mg testosterone propionate (TP) for 7 days and testing started 6 to 7 h after the last injection. A normal spayed female rat made receptive by the above procedure (EB and progesterone treatment) was placed in the observation cage with the test animal. TP treatment of the test subjects was continued for another week at which time another test for masculine sexual behavior was run. At least 2 mounts with pelvic thrusting within 5 min were rated as positive for male sexual behavior.

Results. The normal male and female rats were treated only with homotypical sex hormones, and as expected, displayed the usual dimorphic sexual behaviors[7,8]. The pseudohermaphrodites, who were tested for masculine sexual behavior, and 2 months later for feminine sexual activity, exhibited no sexual response. In fact, none of the pseudohermaphrodites showed the slightest interest in either sexual partner.

Discussion. Regardless of the genetic sex or perinatal endocrine manipulations (castration and/or steroid hormone injections) laboratory animals have always exhibited some sexual behavior as adults[6,8]. Since androgens masculinize sexual behavior[6,8], then the absence of male behavior activity in the rat pseudohermaphrodite can be explained by the animal's androgen insensitivity. However, the absence of feminine sexual behavior in the pseudohermaphrodite is surprising because it does not agree with the generally held concept that feminine differentiation requires no hormonal imprinting. Thus, in the rat pseudohermaphrodite and possibly, also, in the testicular feminized mouse[15] the absence of androgen imprinting is not sufficient to allow for feminine neural differentiation either of reproductive cyclicity[14] or of sexual behavior, but an active feminine organizer appears to be required.

[1] The research was partially supported by a grant from the Rockefeller Foundation. A. S. GOLDMAN is a U.S.P.H.S. Career Development Awardee (No. HD-13,628).

[2] Department of Endocrinpharmacology, Research Laboratories of Schering AG, Berlin, BRD.

[3] A. JOST, Recent Progr. Horm. Res. *8*, 379 (1953).

[4] P. DEMOOR and C. DENEF, Endocrinology *82*, 480 (1968).

[5] C. A. PFEIFFER, Am. J. Anat. *58*, 195 (1936).

[6] K. L. GRADY, C. H. PHOENIX and W. C. YOUNG, J. comp. physiol. Psychol. *59*, 176 (1965).

[7] R. E. WHALEN and D. A. EDWARDS, Anat. Rec. *157*, 173 (1967).

[8] H. STEINBECK and F. NEUMANN, in *Psychopharmacology, Sexual Disorders and Drug Abuse* (Eds. T. A. BAN, J. R. BOISSIER, G. J. GESSA, H. HEIMANN, L. HOLLISTER, H. E. LEHMANN, I. MUNKVAD, H. STEINBERG, F. SULSER, A. SUNDWALL and O. VINAR; North Holland, Amsterdam 1973), p. 503.

[9] B. H. SHAPIRO, A. S. GOLDMAN and A. W. ROOT, Proc. Soc. exp. Biol. Med. *145*, 334 (1974).

[10] C. W. BARDIN, L. P. BULLOCK, R. J. SHERINS, I. MOWSZOWICZ and W. R. BLACKBURN, Recent. Progr. Horm. Res. *29*, 65 (1973).

[11] A. J. STANLEY, L. G. GUMBRECK, J. E. ALLISON and R. B. EASLEY, Recent. Progr. Horm. Res. *29*, 43 (1973).

[12] K. EINARSSON, J.-A. GUSTAFSSON and A. S. GOLDMAN, Eur. J. Biochem. *31*, 345 (1972).

[13] A. S. GOLDMAN, A. W. ROOT, G. DUCKETT and B. H. SHAPIRO, J. Endocr. *64*, 249 (1975).

[14] B. H. SHAPIRO, A. S. GOLDMAN and J.-A. GUSTAFSSON, Endocrinology *97*, 487 (1975).

[15] S. OHNO, L. N. GELLER and E. V. YOUNG LAI, Cell *3*, 235 (1974).

Since the perinatal rat ovary is steroidogenically inactive[16,17] and is not required for feminine differentiation[3–5] it is possible that the rat fetal adrenal which does secrete hormone[3,18,19] and can affect feminine development[9] may organize the brain as female. As to the nature of the hormone, estradiol, like testosterone, prevents development of feminine sexual behavior in either sex of the rat[7,8] but, progesterone antagonizes the masculinizing action of testosterone[20], so that when administered to neonatal male rats it demasculinizes adult sexual behavior[21]. If progesterone is required for the development of a female brain, then the absence of feminine sexual behavior in the rat pseudohermaphrodite can be explained by our demonstration that the adult pseudohermaphrodite does not produce detectable quantities of progesterone[22], and the fact that the animal is also insensitive to progesterone[10]. Furthermore, elevated serum levels of progesterone in the female fetal monkey as compared to the male[23], suggests a possible role for this hormone in female development.

[16] T. Noumura, J. Weisz and C. W. Lloyd, Endocrinology *78*, 245 (1966).
[17] R. J. Schlegel, E. Farias, N. C. Russo, J. R. Moore and L. I. Gardner, Endocrinology *81*, 565 (1967).
[18] T. A. Roos, Endocrinology *81*, 716 (1967).
[19] P. G. Holt and I. T. Oliver, Biochem. J. *108*, 339 (1968).
[20] F. A. Kincl and M. Maqueo, Endocrinology *77*, 859 (1965).
[21] M. Diamond, A. Llacuna and C. L. Wong, Horm. Behav. *4*, 73 (1973).
[22] J.-A. Gustafsson and A. S. Goldman, Endocrinology *90*, 931 (1972).
[23] F. C. Hagemenas and G. W. Kittenger, Endocrinology *91*, 253 (1972).

Plasticity of the Hormone Receptors and Possibility of their Deformation in Neonatal Age

G. Csaba and Susanna U. Nagy

Department of Biology, Semmelweis University of Medicine, Tüzolto utca 58, Budapest IX (Hungary), 24 November 1975.

Summary. Gonadotropin or TSH treated newborn animals reacted to TSH treatment in their adult age in a lesser degree than control ones. This suggests the plasticity of hormone receptors and the possibility of their deformation in neonatal age.

Membrane receptors reacting to vertebrate hormones were found in lower animals also[1–6]. These statements and the fact that in embryonic development, a single cell, the fertilized egg, delivers a great variety of specialized cells having different receptors leads to the conclusion that the receptors also have their own ontogenesis. In our experiments, we tried to elucidate the problem whether these receptors could be influenced or modified at such a critical period of development as the neonatal age.

Newborn Wistar CB rats were s.c. injected – strictly within 24 h after birth – with the following hormones: 1. thyrotropic hormone (TSH) – (Ambinon-Organon, Oss), 1 IU/animal; 2. gonadotropic hormone (Gestyl-Organon, Oss), 100 IU/animal. Animals of the control group were injected with the solvent only. After a lapse of 4 months, the animals were again treated with TSH (3 IU/animal) or, with gonadotropin (50 IU/animal) in a grouping given in the Table. Blood sampling was then made – 30 min after the treatments – by bleeding the animals to death. Concentration of thyroxine in the serum was determined by using radio-immunoassay (Amersham Thyopac-4 kit). Significance of the results was analyzed with the Student's *t*-test.

For examination of the hormone receptor's plasticity, we have chosen deliberately the TSH and the gonadotropin. Namely, these hormones provoke though different effects in adult organisms; they are only related in chemical structure – at least as regards their subunit[7]. Moreover, the gonadotropic hormones are produced only later. We might suppose, on this basis, that large doses of them injected into the newborn could deform the receptor of the chemically related TSH in such a way that its adult reactivity will be changed. For this reason, the change of the thyroxine level served as control of the reaction by determining it after TSH treatment in adult age.

As demonstrated by the results (Table), both the TSH and the gonadotropin treatment of the newborn rats resulted in the treated animals reacting – in adult age – much more weakly to the TSH stimulus than the control ones. The measured differences were significant in all cases. The gonadotropin given to the newborn produced a stronger inhibitory effect than the TSH. More precisely,

Thyroxine level of the serum in the treated and in the control groups

No. of animals	Newborn–adult	Thyroxine (μg/100 ml serum)	Related to the control (%)	Significance related to the control (*p*)
10	Control + TSH	13.54		
8	TSH + TSH	8.17	− 39	< 0.1
9	Gonadotropin + TSH	4.08	− 70	< 0.01
10	Control + gonadotropin	10.43		
9	TSH + gonadotropin	3.39	− 68	< 0.01
9	Gonadotropin + gonadotropin	4.63	− 55	< 0.05
10	Control + NaCl	9.50		
10	Control + TSH	13.54	+ 42	< 0.3
10	Control + gonadotropin	10.43	+ 9	< 0.7

[1] G. Csaba and T. Lantos, Cytobiologie *7*, 361 (1973).
[2] G. Csaba and T. Lantos, Cytobiologie *11*, 44 (1975).
[3] G. Csaba and T. Lantos, Experientia *31*, 1097 (1975).
[4] G. Csaba and J. Bierbauer, Gen. comp. Endocr. *22*, 132 (1974).
[5] G. Csaba and J. Bierbauer, Acta biol. med. germ. *34*, 269 (1975).
[6] G. Csaba, E. Kapa and M. Cserhalmi, Endocrinologie *65*, 219 (1975).
[7] D. N. Ward, in *Invertebrate Endocrinology and Hormonal Heterophylly* (Ed. W. J. Burdette; Springer, Berlin 1974), p. 320.