Effect of Serotonin Deficiency on the Hypothalamic-Pituitary-Gonadal System in Rat Fetuses

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Luteinizing hormone-releasing hormone drops in the hypothalamus of male but not female rat fetuses in serotonin (5-HT) deficiency. This drop is coincident with an increase of the luteinizing hormone (LH) level in blood plasma of males. An influence of testosterone on LH-RH or LH secretion should be ruled out, because the level of testosterone is not changed in the testes and blood plasma.

Key Words: *serotonin; hypothalamus; pituitary gland; luteinizing hormone-releasing hormone*

A large number of investigations devoted to luteinizing hormone-releasing hormone (LH-RH) system, which plays a key role in the neuroendocrine regulation of reproduction, have been undertaken during the last twenty years. Synthesized in neurons of the septo-preoptic region, LH-RH is transported by axons to the medial eminence and released into the hypophyseal portal system of the circulation [4,15]. Finally LH-RH reaches the adenohypophysis, which controls LH secretion. The functional activity of LH-RH neurons is in turn governed by the nervous system including the serotoninergic one from the Raffa nucleus. Serotonin (5-HT) inhibits LH-RH secretion, acting either as a neurotransmitter or as a neuromodulator in adult rats [4,15].

The key role of hypothalamic 5-HT in reproductive regulation in adult animals has prompted scientists to ponder on its possible role in the development of the hypothalamic-pituitary-gonadal axis as well as in sexual differentiation and behavior in ontogenesis [1,2,14]. No data concerning the effect of 5-HT on hypothalamic-pituitary-gonadal activity in the fetus are found in the literature.

In this connection the aim of the present study was to assess the hypothalamic-pituitary-gonadal activity in rats towards the end of embryonal development after chronic inhibition of 5-HT synthesis.

MATERIALS AND METHODS

Pregnant Wistar rats were injected i.p. with the inhibitor of 5-HT synthesis parachlorphenylalanine (PCPA, Sigma, 100 μg/kg) in saline every day from the 8th to the 20th day of gestation (the day of coitus was taken as the 1st day of embryonal life). Control pregnant females received saline only. Fetuses of both sexes were removed from the uterus and decapitated under nembutal anesthesia (50 mg/kg) on the 21st day of development. Thereafter the brain region comprising the anterior and middle hypothalamus along with the septum and diagonal fascicle was dissected. In addition blood samples were collected from fetuses of both sexes and the testes were removed from males and weighed. All tissue samples were frozen in liquid nitrogen and stored at -70°C until analyzed. LH-

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RH was determined in the nerve tissue by the double antibody technique [8]. Synthetic LH-RH (Sigma) was utilized for plotting the standard curve. Antiserum to LH-RH of high specificity conjugated with bovine serum albumin was obtained by rabbit immunization [3]. The antiserum had a high titer (1:1200), providing a minimum sensitivity of the assay at 7.8 ng/ml. Radioiodination of synthetic LH-RH with ^{125}I was performed with iodogen (Serva). Radioactivity of samples was measured with a γ-spectrometer (Searle, Holland) with 90% efficiency of counting for ^{125}I. The intra-assay and interassay coefficients of variation were 7 and 18%, respectively.

Determination of LH in the blood was performed by radioimmunoassay using NIADDK kits which were made available through the Rat Pituitary Hormone Distribution Program. Standard NIADDK-rLH-RP-2 was used as a "cold standard." Radioiodination of NIADDK-rLH-1-6 was performed. Results obtained with the double antibody radioimmunoassay were expressed in ng/ml. The sensitivity of the assay was 0.04 ng/ml.

Determination of testosterone was performed by radioimmunoassay using commercial steron-T-^{125}I kits (Minsk, Belarus). The sensitivity of the assay was 0.2-0.6 ng/ml.

RESULTS

The content of LH-RH was measured in hypothalamic tissue both in males and in females. Reliable sex differences in LH-RH level were not found in control fetuses (Fig. 1). Pretreatment with PCPA resulted in a 3-fold LH-RH decrease in males but not in females under the same experimental conditions.

There were no sex differences in LH content in the blood of control fetuses. The concentration of LH was not changed after PCPA administration to female fetuses. In contrast the same treatment resulted in a weak but significant LH increase in the blood of male fetuses (3.77±0.059 ng/ml in tests vs. 3.50±0.042 in the control) (Fig. 1).

The weight of the body and testes was decreased significantly in fetuses after PCPA treatment (Table 1). This difference was more pronounced in the ratio of testes mass to body mass. The same pharmacological treatment did not result, however, in reliable differences in testosterone content either in the testes or in the in plasma of fetuses.

The approach common in experimental neuroteratology was used in this study [10]. Thus, the inhibition of 5-HT synthesis encompassed the developmental period of its target, the LH-RH system [11,13]. Pharmacological inhibition of 5-HT synthesis has already been successfully employed in studies of the role of this transmitter in the differentiation of target neurons [1,5,6]. These studies showed, that when injected i.p. to pregnant females, PCPA crosses the placental and blood-brain barrier with the blood and inhibits the activity of tryptophan hydroxylase, thereby blocking 5-HT synthesis in the fetal brain [5].

The most expressive index in this study was the marked drop of hypothalamic LH-RH in 5-HT deficiency in male fetuses but not in females.

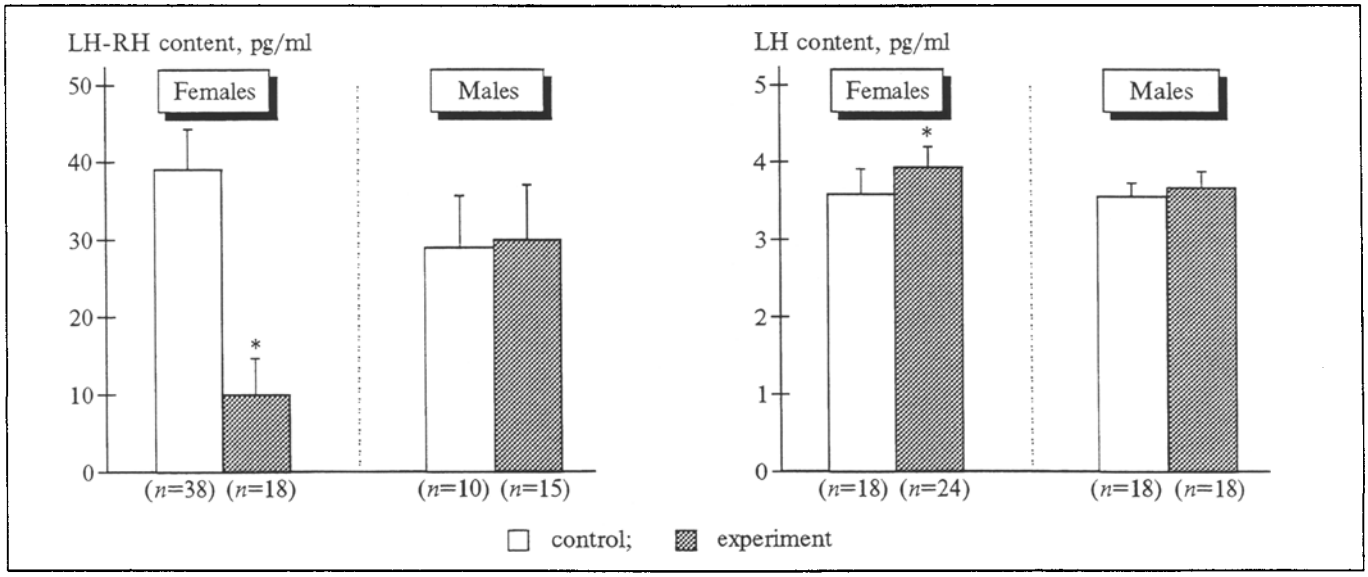

Fig. 1. Effect of PCPA administration to pregnant rats on hypothalamic LH-RH and LH content in blood plasma in male and female fetuses on the 21st day of development. *n*: the number of tests (each sample comprised three brain fragments or blood from 3-4 fetuses). Asterisk denotes $p<0.01$.

TABLE 1. Effect of PCPA Treatment of Pregnant Rats on Body Mass, Testis Mass, and Testosterone Content in Blood Plasma and Testes of Fetuses on the 21st Day of Development ($M \pm m$)

Experimental conditions	Body mass, g	Testis mass, mg	Testosterone content	
			in testes, ng/mg	in plasma, ng/mg
Control	3.82±0.08 (22)	3.24±0.15 (24)	1.05±0.02 (10)	0.81±0.84 (5)
PCPA	2.93±0.07* (24)	2.71±0.13* (27)	0.91±0.13 (10)	1.03±0.40 (6)

Note. The number of tests is given in parentheses; asterisk denotes $p<0.01$.

The simultaneous LH increase in the blood of males indirectly attests that the LH-RH drop is due to increased release rather than inhibition of its synthesis and that 5-HT inhibitory control of gonadotropic function sets in at least by the 21st day of embryonal life, but only in males.

We cannot account for the mechanism of sex differences in LH-RH and LH responses to chronic inhibition of 5-HT synthesis. Evidently, they do not have to do with the activating (reversible) effect of sex steroids. In fact, chronic pretreatment with PCPA does not change the level of testosterone (Table 1) and estradiol [1] in blood plasma. Nevertheless, these differences are probably related either to the prenatal masculinizing (irreversible) effect of testosterone on the brain [12] or to genetic sexual characteristics inherent in developing hypothalamic neurons [7]. For example, testosterone was found to masculinize the distribution of serotoninergic fibers in the medial preoptic nucleus, decreasing their density in fetuses and to a lesser extent in newborns [9].

Thus, chronic inhibition of 5-HT synthesis presumably stimulates hypothalamic-pituitary-gonadal activity in male fetuses but does not affect females.

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