ELEVATION OF THE HYPOTHALAMIC NORADRENALIN LEVEL IN NEWBORN FEMALE RATS BY THE ACTION OF 4-HYDROXYESTRADIOL-17β

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UDC 612.826.4.018.577.175.523].014.46:[615.357:577.175.642]-053.31.019:599.323.4

KEY WORDS: catecholesterogens; catecholamines; hypothalamus; sexual differentiation

Sexual differentiation of neuroendocrine mechanisms of reproduction takes place under the influence of androgens and their active metabolites: catecholesterogens (CE), formed as a result of consecutive reactions of aromatization and hydroxylation [4, 10]. The writers previously obtained proof of a functional link between metabolic aromatization of testosterone (T) and elevation of the catecholamine (CA) level induced by it in the hypothalamus of newborn female rats [3]. There is evidence that CE can raise the CA concentration in the adult rat brain as a result of competition for active sites of catechol-O-methyltransferase (COMT), one of the principal enzymes of CA catabolism [5, 6]. Data [2, 11] on mediation by CE of the action of the androgen on the developing brain and qualitative differences discovered in this way between the physiological effects of 2- and 4-hydroxy isomers of CE suggest that 4-hydroxyestradiol-17β (4-OH-E_2) is an inducer of CA accumulation. The aim of this investigation was to study this problem.

EXPERIMENTAL METHOD

Experiments were carried out on newborn male Wistar rats. 4-OH-E_2 (generously provided by Professor R. Knuppen, West Germany, and the World Health Organization, "Steraloids," USA) was dissolved in propylene-glycol with 0.01% ascorbic acid and injected subcutaneously in a dose of 10 μg daily from the lst through the 5th days of life (the day of birth was taken to be the 1st day of life). Control animals received injections of the solvent. Concentrations of noradrenalin (NA) and dopamine (DA) in the hypothalamus were determined spectrofluorometrically [7] 24 h after the last injection of the preparation, and also on the 7th, 10th, and 12th days of life. The significance of differences was calculated by Student's test.

EXPERIMENTAL RESULTS

A significant increase was found in the NA concentration in the hypothalamus of the female rats 24 h after the last injection of 10 μg 4-OH-E_2, given for the first 5 days of postnatal life (Fig. 1). The NA concentration in the experiment was 5.50 $\pm$ 0.62 nmole/g tissue, and in the control 3.73 $\pm$ 0.45 nmole/g tissue ($p < 0.05$). The DA level was unchanged under these circumstances.

The NA and DA concentrations on the 7th, 10th, and 12th days of life did not differ from the control values.

The results are in agreement with those of previous investigations [1, 3] which showed an increase in the CA concentration in the hypothalamus of newborn female rats under the influence of exogenous T. The increase in the NA concentration in the hypothalamus under the influence of 4-OH-E_2 coincides with the time of maximal CE accumulation in the brain structures during parenteral administration [9].

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Presented by Academician of the Academy of Medical Sciences of the USSR Yu. A. Pankov. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 6, pp. 555-556, June, 1990. Original article submitted February 3, 1989.

0007-4888/90/0006-0738$12.50 ©1990 Plenum Publishing Corporation

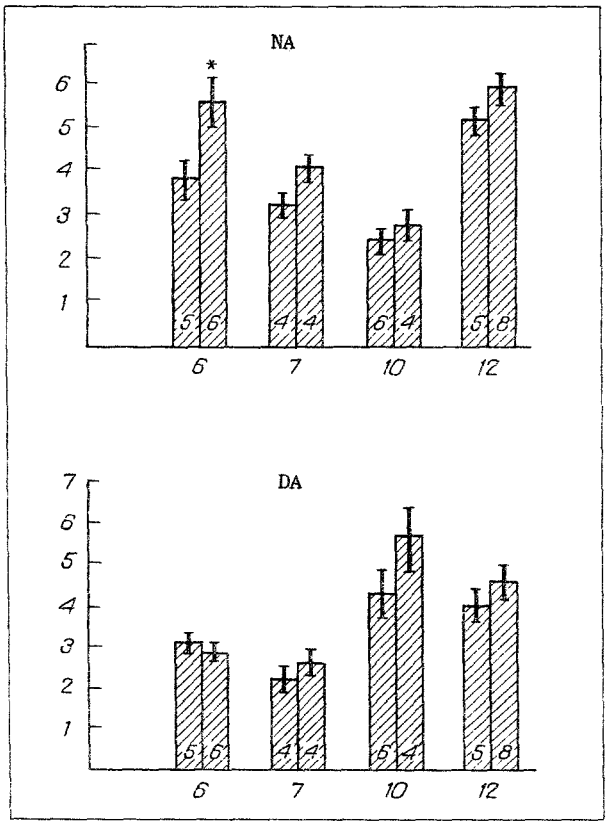

Fig. 1. Effect of injections of 4-OH-E_2 from the lst through the 5th day of life on NA and DA concentrations in hypothalamus of newborn female rats. Abscissa, days of postnatal life; ordinate, concentrations of NA and DA (in nmoles/g tissue). Columns on left are control, on right, experiment. Numbers in columns indicate number of analyses. *$p < 0.05$ compared with control.

Evidence of the high defeminizing ability of 4-OH-E_2, simulating the effects of neonatal androgenization in relation to sexual differentiation of the hypothalamus was obtained by the writers previously [2]. The high effectiveness of 4-OH-E_2 as an inducer of defeminization has been noted by other workers also [8, 11]. If these facts are compared with the results of the present investigation, it can be concluded that a correlation exists between the raised hypothalamic NA level of newborn female rats induced by 4-OH-E_2 and the defeminizing effect of CE.

One possible explanation of the highly effective action of 4-OH-E_2 is the ability of CE to act on receptors for esterogens in the developing rat brain. A marked increase in concentration of receptors for esterogen has been found [11] 24 h after injection of 10 μg of 4-OH-E_2. However, the authors cited were unable to establish correlation between binding with receptors and the defeminizing effect of CE, for 2-hydroxyestradiol-17β, which has no defeminizing effect, caused a similar rise of the concentration of receptors 2 h after its administration.

These findings confirm the hypothesis that CE, by inhibiting COMT, establish a direct biochemical connection in the developing brain between sex steroids and neurotransmitters [4].

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DETECTION OF MYELOPEROXIDASE IN HUMAN EYE TISSUE

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UDC 612.84.015.11:577.152.193.088.1

KEY WORDS: myeloperoxidase; lens; retina; eye

Myeloperoxidase (MPO; EC 1.11.1.7) is an enzyme which is usually detected in peripheral blood neutrophils [5, 9]. Its main function is to produce the bactericidal hypochlorite iom (ClO^-) by interaction between hydrogen perioxide and chlorine ions [4]. Unlike the various metabolites constantly found in the animal body, ClO^- is a powerful oxidizing agent [2]. The presence of MPO in the eye tissues has not previously been discussed in the literature, although there are certain facts which may suggest that this is so. For instance, it has been shown that the retina possesses phagocytic properties, without which it would be impossible today to imagine regeneration and the working of the photoreceptor apparatus of the eye [11]. The more superficial parts of the eye are "armed" against infectious agents in the following way: the secretions of the lacrimal fluid contain lysozyme and the aqueous humor contains hydrogen peroxide [6]. However, there are no data on the presence of myeloperoxidase activity in the eye tissues. The investigation described below was carried out to study this problem.

EXPERIMENTAL METHOD

Eyes from previously healthy individuals dying from chest injury or alcohol poisoning and eyes from persons with senile cataract were used for the investigation. The lenses were extracted from the eye and kept until required for analysis at −8°C. The various eye structures were homogenized in 0.15 M NaCl solution. Enzyme activity was determined with *o*-dianisidine reagent at 20°C by the standard method [7]. For this purpose, to 50 μl of extract or MPO ($0.9 \cdot 10^{-8}$ M) was added 500 μl of phosphate buffer, pH 6.0, containing 0.6 mg/ml of *o*-dianisidine hydrochloride. Specific MPO inhibitors, quercetin and morin [10], were used in a concentration of 10^{-4} M. The velocity of the MPO enzyme reaction was measured during the first 30 sec after formation of the reaction mixture, by determining the formation of the product staining at 460 nm. Next H_2O_2 was added to the cuvette up to a final concentration of $1.4 \cdot 10^{-4}$ M. The appearance of a color was recorded on a "DU-7" spectrophotometer (Beckman, Austria). MPO isolated from human blood leukocytes as in [3] was used as enzyme for calibration in the enzyme test. The ratio between coefficients of absorption at 430 and 280 nm, which gives a measure of the degree of purity of the enzyme [12], was 0.60 and enzyme activity according to the *o*-dianisidine test was 200 units/mg.

For immunochemical identification of MPO in the tissue extract, the immunodiffusion test in gel was carried out by the method in [8] in the modification in [1]. A monospecific antiserum to human MPO, obtained by standard immunization of rabbits, was used.

Research Institute of Physicochemical Medicine, Ministry of Health of the RSFSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR Yu. M. Lopukhin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 6, pp. 557-558, June, 1990. Original article submitted July 20, 1989.

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