Sex-Related Peculiarities of Acetylcholinesterase Activity in the Dorsal Nucleus of the Vagus in Newborn Rats

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 121, No. 5, pp. 594-596, May, 1996
Original article submitted February 17, 1995

Acetylcholinesterase activity was examined histologically and enzymatically in the rostral, middle, and caudal parts of the dorsal nucleus of the vagus in newborn rats of both sexes in the critical period of sexual differentiation of the brain. It was found that the amount of active neurons in the middle and caudal parts depends on sex, i.e., it was reliably greater in the dorsal nucleus of males than of females on the 7th day after birth.

Key Words: *acetylcholinesterase; ontogenesis; sexual differentiation; dorsal nucleus of the vagus; carbohydrate metabolism*

Our attention was focused on the dorsal nucleus of the vagus (DNV) during long-term experimental investigation of whether there is a monosynaptic nervous connection between the neurons of the paraventricular nuclei of the hypothalamus and DNV neurons [1]. Our findings as well as the data of other laboratories [11,13,14] permitted us to postulate that there is a nerve-conducting pathway that connects neurons of the paraventricular hypothalamic nuclei with the pancreatic islets via the vagus nerve. By this pathway the hypothalamus may control the endocrine function of the pancreas and carbohydrate homeostasis [3]. According to this concept, the paraventricular-vagal system plays a specific role in realizing the sex-dependent mechanisms of carbohydrate homeostasis control in the overall ensemble of sources and pathways of regulation of carbohydrate metabolism [2]. Experimental data validating this concept concerned one component of the regulatory system, the hypothalamic paraventricular nuclei [2].

The present investigation was undertaken to seek evidence for the sexual dependence of another regulatory component, the DNV. We based our premise on the common notion that sex-dependent brain structures are influenced in early ontogenesis by sex hormones, particularly by androgens (what is known as hormonal imprinting). This imprinting results in sexual differences in the structural and functional organization of sex-dependent brain structures. The phenomenon of hormonal imprinting is realized during the first 10 days after birth (the critical period of sexual differentiation), and therefore we proposed seeking histophysiological manifestations of sexual dimorphism in DNV neurons precisely during this period, particularly during the first week, which is considered to be the most sensitive to sex hormones. There are published data on the direct effect of sex hormones on the brain cholinergic structures [7]. In addition, it has been shown that the increase of acetylcholinesterase activity correlates with the period of neuron differentiation [5] and of active synaptogenesis [12], which is stimulated by sex hormones [9]. In view of this, acetylcholinesterase (ACE) activity was studied histochemically in developing neurons of the DNV in rats of both sexes during the first week after birth.

MATERIALS AND METHODS

For the study newborn rats of both sexes aged 3, 5, and 7 days and mature 2-month-old male Wistar

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0007-4888/96/0005-0537$15.00

rats were used. The animals were killed by decapitation. The brain was removed from the skull and then frozen with dry ice. Histochemical study of ACE was performed using a modified method [15] on 12-μ fresh-frozen cryostat frontal sections of the medulla oblongata fixed in neutral formalin for 15 min. For easy identification of neurons with no or a very low reaction all sections were additionally stained with aluminous carmine. The reaction in DNV neurons was analyzed by a semiquantitative method, and the intensity of the reaction in neuron bodies was assessed visually according to a 5-point scale (absence of reaction, very low, low, moderate, and high reactions). Cells with different types of reaction were counted on every 10th section in six positions, namely in the rostral, middle, and caudal parts of the rostrocaudal extent of the DNV individually for the left and right sides. The summed data for each position were divided by the number of sections used. Then the mean values of the cell content characterizing the five types of reaction were expressed in percent, the total number of cells on an averaged section being taken as 100%. The data in percent were processed statistically using the Student *t* test.

RESULTS

The reaction was negative or very low in the majority of animals in the early postnatal period. With age the number of cells with moderate and high enzymatic activity increased in all parts along the rostrocaudal extent of DNV. It should be stressed that the total number of DNV neurons with or without an ACE reaction is not related to the animal's sex and in all age groups of newborn rats is comparable with the number in mature animals on the 7th day of life. A histochemical reaction was found both in large (more than 15 μ in diameter) and in small neurons. High and moderate reactions were noted predominantly in large neurons. In all age periods active cells were most numerous in the middle part, then somewhat fewer in the caudal, and fewest in the rostral part of the DNV (Fig. 1). There were no cells with high or moderate enzymatic activity on the 3rd day after birth either in males nor in females. They appeared on the 5th day in the middle and caudal parts, exhibiting predominantly a moderate reaction. A high intensity of reaction was found only in males in single neurons localized in the middle part of the DNV (Fig. 1). On the 7th day the number of cells with a moderate or high reaction was greatly increased but still significantly lower (2-3 times lower in the caudal and middle parts and 5-6 times lower in the rostral part) than in mature animals. This increase in the caudal and middle parts was more marked in females than in males; moreover, cells with a high intensity of reaction were found in all parts of the nucleus in males but only in the middle part and in a smaller number in females (Fig. 1). Cells with high and moderate ACE activity were mostly found in the middle of the DNV and comprised a little more than 50% of the total number of cells at this level of the rostrocaudal extent of the nucleus (Fig. 1), the number of cells with a high reaction being nearly 1.5-fold greater than the number of cells with a moderate reaction. Active cells (with high or moderate reaction) comprised 38% (most of them with moderate activity) in the caudal part of the DNV of mature males, while in the rostral part the number of cells with a high or moderate reaction amounted to 33% of the total number of cells, 15% of them being cells with high enzymatic activity.

Unlike other paired nuclei of the cranial nerves, the DNV is known to manifest asymmetry, because its left half innervates predominantly the peritoneal organs [6]. There were no significant differences in enzymatic activity between the left and right sides of the DNV in the age period studied.

Analyzing the histological and enzymatic data, we may assume that even on the 7th day after birth,

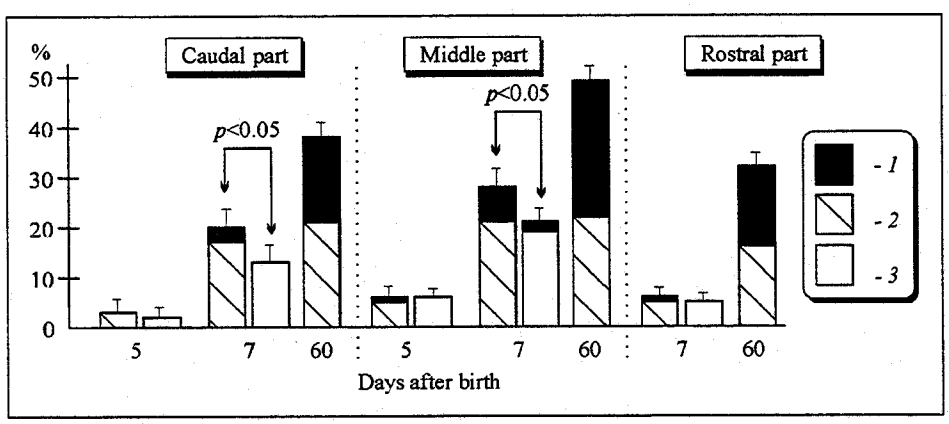

Fig. 1. Number (in % of total amount) of neurons with high (*1*) and moderate (*2* — males and *3* — females) activity of ACE in caudal, middle, and rostral parts of DNV in newborn and mature rats.

when the number of active cells rises dramatically, it is still much lower than in mature animals (Fig. 1). At the same time, the number of cells with high or moderate enzymatic activity in this age group (7 days) shows a sex dependence, namely the number of cells with a high or moderate ACE reaction in the caudal and middle parts of the DNV is reliably higher in males than in females. Moreover, neurons with high enzyme activity appeared in males earlier (5th day) and in greater numbers (7th day) than in females. Thus, the biochemical maturation of dorsal nuclei of the vagus tested by the histochemical ACE reaction is not complete by the 7th day of life. Nevertheless, there is no doubt that these processes occur more rapidly in males than in females in the early postnatal period. It is also evident that the maturation of cholinergic structures is different along the rostrocaudal extent of the DNV. These rostral structures form later than the caudal and middle parts. Since the sex steroid-sensitive regions of the brain of males, unlike that of females, are under the influence of testosterone in early ontogenesis [8], our findings agree with the published data on the premature biochemical maturation of the brain induced by exogenous sex hormones in early ontogenesis [4]. They also correlate with our previous data on the existence of testosterone-metabolizing enzymes in the studied brain region [10]. Thus, it may be thought that the DNV is sensitive to the circulating sex steroids. This sensitivity probably underlies its sexual differentiation that is accomplished in early ontogenesis. Hence, the findings of this study may provide further evidence that the sexual affiliation of neurons of the paraventricular-vagus pathway at both levels of its organization, hypothalamic and medullary, underlies the mechanisms which regulate the sex-dependent peculiarities of carbohydrate metabolism.

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