PHYSIOLOGY

Structural Characteristics of Adenohypophysis in Hypertensive ISIAH Rats in Early Ontogeny

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Comparative morphological study of the adenohypophysis was conducted in 3-week-old normotensive WAG and hypertensive ISIAH rats (prehypertension period) to elucidate the role of the adenohypophysis in the development of essential hypertension. Morphometric analysis revealed ultrastructural signs of functional activation of somatotrophs, gonadotrophs, and corticotrophs in ISIAH rats. These peculiarities of structural organization of adenohypophysis in hypertensive rats can attest to enhanced response of the hypothalamic-pituitary-adrenal axis in these animals to natural stress associated with their transition to independent feeding. Increased stress sensitivity during the prehypertensive period of postnatal ontogeny contributes to the development of arterial hypertension.

Key Words: *ISIAH rats, adenohypophysis; somatotrophs; gonadotrophs; corticotrophs; thyreotrophs*

ISIAH rats (inherited stress-induced arterial hypertension) are a genetic model of stress-sensitive arterial hypertension [3]. Previous morphological studies revealed hyperplastic changes in the adrenal cortex and medulla in adult ISIAH rats [2]. Moreover, these animals demonstrated signs of zona fasciculata cells hyperplasia in young rats (3 weeks) before hypertension onset [1], which indicated the activation of the hypothalamic-pituitary-adrenal axis (HPAA). Perhaps, ISIAH rats at this age show an increased HPAA response to stress associated with the transition to independent feeding. Adenohypophysis is an important regulatory element of endocrine function of HPAA. In light of this, we studied the structure of the adenohypophysis in normotensive WAG and hypertensive ISIAH rats during "critical" period of postnatal ontogeny associated with cessation of milk feeding.

MATERIAL AND METHODS

Experiments were performed on 3-week-old male hypertensive ISIAH rats (experiment) and normotensive WAG rats (Wistar albino Glaxo; control). The work was conducted in accordance with "Rules of the work using experimental animals" (Annex to Decree of the USSR Ministry of Health No. 755 of 12.08.1977). ISIAH rats had higher body weight (34.60±0.76 g, $p<0.05$) than WAG rats (28.40±1.06 g). In anesthetized animals (ether anesthesia), pituitary was isolated after decapitation and placed in a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffered saline at 4°C for 4 h. Fixed pituitary was cut

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into pieces and left in the buffer overnight. Tissue samples were postfixed in 1% OsO_4 (1.5 h), dehydrated in alcohols and propylene oxide, and embedded into a mixture of epoxy resins (epon and araldite). Semithin sections were examined under a Jem-1400 electron microscope (Jeol). Micrographs were obtained with a digital camera Olympus Veleta. Morphometrical analysis of several types of adenocytes was performed using iTEM software (Olympus), cross-sectional area of adenocytes and their nuclei, diameter of secretory granules and their numerical density, relative volumes of mitochondria, rough endoplasmic reticulum (EPR), and Golgi apparatus were evaluated.

The variables were analyzed and checked for normality using Statistica 6.0 software. Quantitative data are presented as mean and error of the mean ($M \pm m$). The significance of differences was evaluated by Student's t test, the differences were significant at $p<0.05$.

RESULTS

Adenohypophysis of 3-week-old WAG and ISIAH rats was a differentiated endocrine gland containing adenocytes of different functional specialization: somatotrophs, gonadotrophs, corticotrophs, and thyreotrophs. Among the adenocytes, somatotrophs usually prevailed quantitatively. In WAG rats, most somatotrophs had dense cytoplasm with a large number of secretory granules 0.20-0.30 μ in diameter and well developed EPR and Golgi apparatus. In ISIAH rats, the population of somatotrophs was more heterogeneous due to the presence of large light cells among the dense somatotrophs. Mean cross-sectional area of somatotrophs in hypertensive animals was greater than in normotensive rats (Table 1). Large cells had wide channels of EPR and Golgi apparatus, their cytoplasm contained numerous "dissolving" secretory granules with moderate electron density with interrupted membrane or without it (Fig. 1). At the same time, the number of electron-dense granules was lower, their numerical density and volume fraction in ISIAH rats were below the control values (Table 1). Volume fractions of mitochondria, EPR and Golgi complex did not differ significantly in rats of various strains. Enhanced degranulation of somatotrophs in hypertensive rats can be related to increased stimulation of somatotropin release; the amount of this hormone is known to determine the growth rate. Indeed, ISIAH rats had lower body weight at birth, at the age of 2 weeks they lagged behind normotensive rats by this parameter, but then began to grow faster and by the age of 3 weeks the body weight of hypertensive rats surpassed that of age-matched normotensive rats by almost 20%. Published data suggest, that animals with low body weight at birth are characterized by increased HPAA activity [10].

TABLE 1. Morphometrical Parameters of Adenocytes in Adenohypophysis of 3-Week-Old Rats

Type of adenocyte	Rat strain	Area, μ²		Numerical density of secretory granules, μ^{-2}	Relative volume, %			
		cells	nuclei		secretory granules	mitochondria	EPR	Golgi apparatus
Somatotrophs	WAG	39.20±3.53	16.10±2.35	3.80±0.72	16.10±2.16	6.08±1.26	7.83±2.63	8.79±1.24
	ISIAH	67.20±11.22*	20.00±4.09	2.50±0.37*	10.6±1.8*	4.40±0.84	11.70±1.73	11.2±1.7
Corticotrophs	WAG	57.90±7.56	18.00±1.88	7.10±1.11	9.50±1.04	8.50±1.14	7.60±1.15	7.00±1.41
	ISIAH	58.00±6.82	17.40±3.27	3.60±0.41*	6.30±0.86*	5.90±0.63*	15.50±3.89*	6.00±0.88
Gonadotrophs	WAG	42.50±3.81	15.90±1.39	7.90±0.92	12.20±0.61	7.30±0.77	6.10±0.82	5.90±0.86
	ISIAH	68.50±7.09*	25.50±3.02*	2.40±0.62*	6.60±0.87*	5.40±0.93	11.80±2.37	4.50±0.92
Thyreotrophs	WAG	41.70±4.02	18.40±2.23	4.7±1.0	5.70±1.62	6.10±1.42	6.00±0.89	5.5±1.2
	ISIAH	47.60±5.36	18.00±1.86	3.70±0.61	5.70±1.09	6.50±0.83	11.40±1.86*	6.50±1.14

Note. *$p<0.05$ compared with WAG.

Corticotrophs in rats of the studied strains had stellate shape, eccentrically located nuclei, and secretory granules 0.10-0.20 μ in diameter (average 0.120± 0.003 μ) arranged along the plasma membrane. Rough EPR cisterns were localized around the nucleus in rows, compact Golgi apparatus was not always clearly seen, although the extent of development of these organelles varied in different cells depending on functional activity. Comparison of the studied strains demonstrated more pronounced cytoplasmic heterogeneity of these cells in ISIAH rats, although they did not differ from corticotrophs of WAG rats in size, shape, and diameter of secretory granules. In hypertensive animals, significant number of corticotrophs typically had light cytoplasm with a small amount of secretory granules, considerably dilated EPR channels and cisterns of the Golgi apparatus, and swollen mitochondria (Fig. 2). In some cases we observed the release of secretory granules from cell cytoplasm into the extracellular space through the damaged plasma cell membrane. Morphometry of corticotrophs in hypertensive animals revealed significant decrease in numerical density and volume fraction of secretory granules, reduced volume fraction of mitochondria, and increased volume fraction of EPR (Table 1). Migration of some corticotrophs into the pericapillary space was considered as a sign of activation (Fig. 3). Structural features of corticotrophs in hypertensive rat indicate increased functional stimulation. Hyperplastic changes of adrenal adrenocortical cells detected in 3-week-old ISIAH rats also confirm enhanced corticotropic effects of adenohypophysis [1]. It should be noted that at the age of 3 weeks the rat pups experience stress related to transition to independent nutrition [2]. In this case, ISIAH rats were more stress-sensitive than WAG rats. ISIAH rats show increased transcriptional activity of proopiomelanocortin gene, enhanced expression of this gene is a cause of increased secretory activity of corticotrophs [7]. In young SHR rats, reduced expression of proopiomelanocortin mRNA was found as well as decreased numerical density of antepituitary corticotrophs (by 30%) in comparison with normotensive Wistar rats [9]. In a study performed on adult SHR rats, changed ACTH response to corticoliberin was observed, which led to stimulation of the adrenal cortex and changes in HPAA function in hypertensive animals [11]. It cannot be excluded that in ISIAH rats mechanisms underlying increased morphofunctional activity of corticotrophs may be different. In a previous work, overexpression of the corticotropin-releasing hormone gene in hypothalamic nuclei of ISIAH rats was revealed. This hormone is the main stimulator of ACTH-secreting cells in adenohypophysis and its increased level can be the cause of enhanced activity of corticotrophs [5,6].

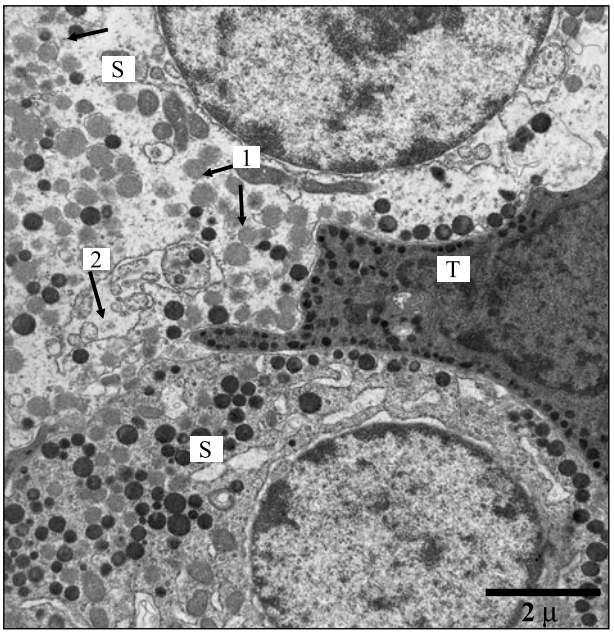

Fig. 1. Adenohypophysis of 3-week-old ISIAH rats. Somatotrophs (S) contain a lot of "dissolving" secretory granules (arrows) and widened cisterns of EPR. Thyreotroph (T), ×12,500.

Gonadotrophs in 3-week-old male rats were characterized by larger size and elongated shape and contained secretory granules of different size and shape. About 70% secretory granules were 0.05-0.15 μ in diameter and the rest 0.15-0.25 μ. In ISIAH rats, cells of larger size with swollen cytoplasm were detected among the gonadotrophs. Cross-sectional area of the cells and their nuclei were larger than in controls (Table 1). Gonadotrophs contained lower number of secretory granules, their relative volume and numerical density

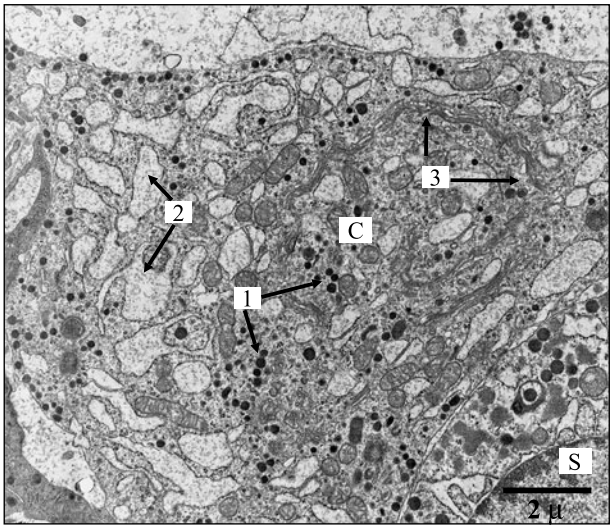

Fig. 2. Adenohypophysis of 3-week-old ISIAH rats. Corticotroph (C) contains a low number of secretory granules (*1*), widened EPR (*2*), and cisterns of Golgi apparatus (*3*). Somatotroph (S), ×10,000.

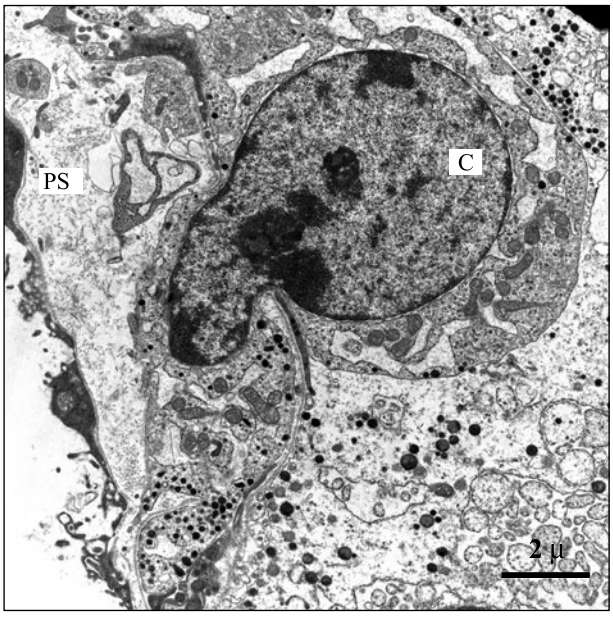

Fig. 3. Adenohypophysis of 3-week-old ISIAH rats. Migration of a corticotroph (C) into the pericapillary space (PS), ×10,000.

were also significantly lower than in WAG rats. It can be assumed that reduced content of secretory granules in gonadotrophs, somatotrophs, and corticotrophs is due to their accelerated excretion. At the same time, the relative volumes of mitochondria, EPR, and Golgi apparatus did not differ from the control values. It was shown that basal plasma levels of testosterone in adult ISIAH rats is higher than in normotensive animals [4].

Thyreotrophs are another type of adenocytes. In WAG rats, these cells typically contained small (0.07-0.12 μ in diameter) secretory granules scattered in the cytoplasm. In thyreotrophs, certain heterogeneity was noted in the density of cytoplasmic matrix, structure of mitochondria, Golgi apparatus and rough EPR depending on cell functional state. Morphological parameters of thyreotrophs in hypertensive and normotensive rats did not differ significantly, except for the fact that in ISIAH rats these cells had increased volume fraction of EPR (Table 1), which can be considered as an initial sign of increased functional activity of thyreotrophs at this developmental stage. Increased blood thyroxine levels in adult ISIAH rats supports this assumption [8].

Thus, structural peculiarities of adenocytes (mainly somatotrophs, gonadotrophs and corticotrophs, and to a lesser extent, of thyreotrophs) revealed in 3-week-old ISIAH rats and indicating functional activation of the adenohypophysis can be regarded as an evidence of increased HPAA response of these animals to natural stress associated with their transition to independent feeding. Higher sensitivity to stress in ISIAH rats during the prehypertensive period of postnatal ontogeny contributes to the development of arterial hypertension.

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