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Cardiac Natriuretic Peptides and Development of Hereditary Hypertension in Rats

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Ultrastructure of the right atrial cardiomyocytes of suckling ISIAH rats was studied to clarify the role of cardiac natriuretic peptides in hypertension development during the period when blood pressure is not yet elevated. Cardiomyocytes diameter was significantly greater, Golgi complex was more developed, and granules in the sarcoplasm were more abundant in ISIAH rats as soon as on postnatal day 12 in comparison with age-matched normotensive animals. The smaller diameter of granules and their qualitative composition (ratio of forming, mature, and dissolving forms) attest to active synthesis and release of secretory product. In 21-day-old ISIAH rats, granule size and qualitative composition reflected increased accumulation of hormones in the cells. Thus, morphological features of increased production of natriuretic peptides in the right atrial myocytes were revealed in rats during the first postnatal month before manifestation of hereditary hypertension.

Key Words: *natriuretic peptides; hypertension; heart; rat*

The main physiological effects of cardiac peptides are regulation of blood pressure and maintenance of water and salt homeostasis via stimulation of natriuresis and diuresis and reduction of peripheral resistance. Their main and indirect effects come down to the antagonism with the renin-angiotensin-aldosterone system. Natriuretic peptides are synthesized in atrial myocytes, accumulated in the secretory granules, and released into the circulation upon mechanical stretching of the myocardium under conditions of increased volume or pressure load [8]. Rats with inherited stress-induced arterial hypertension (ISIAH) were bred at the Institute of Cytology and Genetics, Siberian Division of the Russian Academy of Medical Sciences [2]. At the age of 6 months, these animals exhibit ultrastructural signs of increased secretory activity compared with normotensive controls against the background of high hypertension (~180 mm Hg at rest) and myocardial hypertrophy [1]. Hypertension and left-ventricular hypertrophy in ISIAH rats are registered from the second postnatal month [3,5]. The involvement of natriuretic cardiac peptides in the mechanisms underlying hereditary hypertension in both clinical and experimental studies has not been clearly understood [9,10].

We studied ultrastructure of the right atrial cardiomyocytes of ISIAH rat pups for evaluation of their secretory apparatus during the milk feeding period when blood pressure is not yet elevated.

MATERIALS AND METHODS

ISIAH and WAG rats (normotensive controls) were kept under standard vivarium conditions with unlimi-

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ted access to water and food. Rat pups (n=6 per group) were taken from dams on postnatal days 12 and 21 and sacrificed with a guillotine under ether anesthesia in accordance with the Rules of the Work Using Experimental Animals and European Community Council Directive of 24 November 1986 (86/609EEC). The right atrium was fixed in 2.5% glutaraldehyde and 2% paraform, post-fixed in 1.5% OsO_4, and embedded in a mixture of epon and araldite for electron-microscopic study. Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined under a Jeol JEM-1400 electron microscope. Relative volumes of organelles in the cytoplasm of cardiomyocytes, numerical density of secretory granules and their diameter were determined in at least ten random fields of view in each sample at ×10,000 using a square test grid with 1 μ spacing (100 points) and iTEM 5.1 software (Olympus).

The data were statistically processed and checked for normality using Statistica 6.0 software. The results were presented as mean and error of the mean (M±m). The significance of differences from the controls was determined by Student's t test at p<0.05.

RESULTS

The relative heart weight and the diameter of right atrial cardiomyocytes in ISIAH rats were significantly higher than in WAG rats as soon as on at postnatal day 12 (Table 1). The number of myofibrils and mitochondria did not differ in rats of both strains. However, the relative amount of secretory structures (Golgi complex and granules) more than 2-fold surpassed that in hypertensive rats in comparison with age-matched controls, and free glycogen content in the cytoplasm was lower. The detailed morphological and morphometric study of the secretory granules showed that not only the quantity, but also the quality of the granules was significantly different from the control. For convenience of the analysis, three basic morphological types of granules with the transitional forms between them were discriminated. Mature granules had precisely round shape with distinct boundary membrane and almost homogeneous electron-dense content. Dissolving granules had no clear-cut boundary membrane; their edges looked blurred and jagged in section; heterogeneous core had low density. Electron-dense substances with no clear boundaries within the expanded cisterns of the Golgi complex were regarded as forming granules. Small granules with a clear bright rim under the membrane localized in Golgi region were also assigned to this group (Fig. 1, *a*, *b*). This classification proved to be quite informative and was used in morphological studies [4,6].

Measurement of a large number of granules (about 250 in each group) showed that their mean diameter

TABLE 1. Parameters of Cardiomyocytes of the Right Atrium in ISIAH and WAG Infant Rats

Parameter	Age of rats			
	12 days		21 day	
	WAG	ISIAH	WAG	ISIAH
Heart weight/body weight, mg/g	5.20±0.33	7.70±0.38*	5.00±0.24	5.50±0.03
Cardiomyocyte diameter, μ	6.00±0.09 (269)	7.3±0.1* (277)	6.0±0.1 (216)	7.40±0.15* (178)
Relative volume, %	(60)	(60)	(60)	(61)
myofibrils	47.90±0.38	47.10±0.62	51.20±0.41	50.90±0.42
mitochondria	36.8±0.4	35.50±0.64	39.50±0.42	35.90±0.56*
glycogen	4.50±0.17	3.00±0.17*	1.50±0.15	1.50±0.14
Golgi complex	1.70±0.22	3.70±0.28*	1.70±0.14	3.70±0.21*
granules	1.10±0.13	2.8±0.2*	1.30±0.11	3.30±0.19*
other	8.00±0.29	7.85±0.31	4.80±0.27	4.7±0.3
Diameter of the granules, nm	229.0±5.7 (293)	211.0±4.4* (247)	227±3 (293)	251.0±3.2* (270)
mature	230.0±4.5 (133)	255.0±4.9* (75)	250.0±3.9 (111)	274.0±4.1* (135)
dissolving	223.0±7.6 (87)	189.0±3.9* (93)	227.0±4.4 (129)	240.0±3.9* (104)
forming	148.0±5.9 (55)	174.0±4.5* (74)	179.0±5.7 (53)	176.0±6.3 (31)
atypical	496±26 (18)	520±33 (5)	-	547±43 (7)

Note. In parentheses: number of measurements. *Interstrain differences are significant at p<0.05.

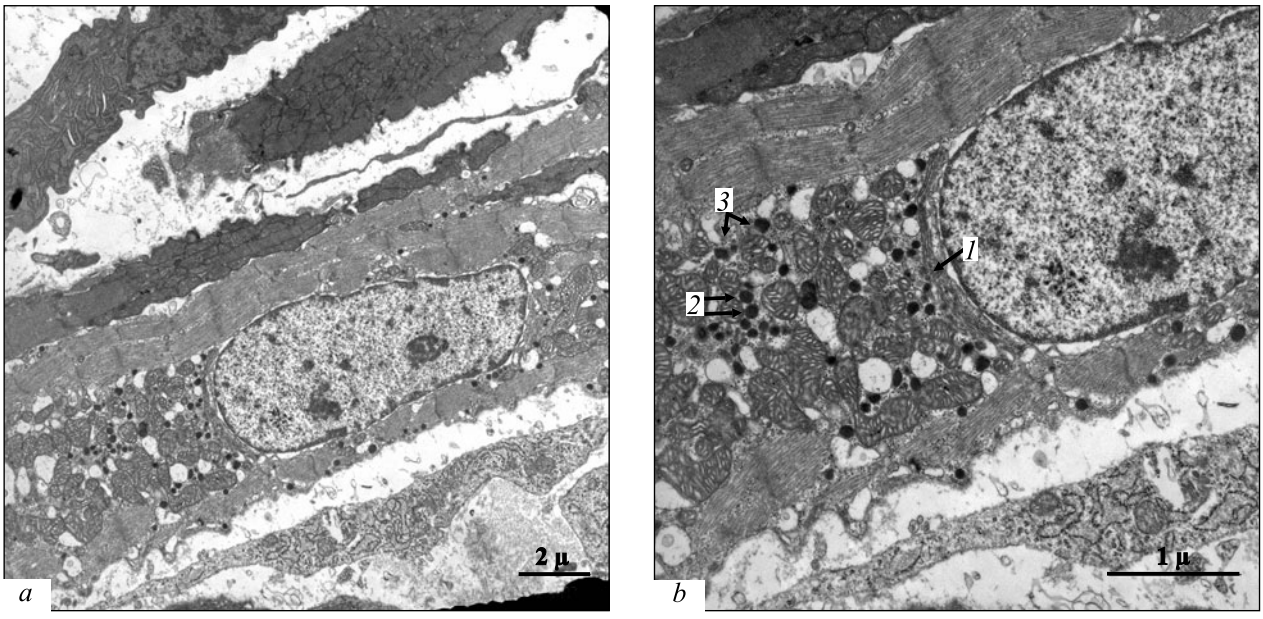

Fig. 1. Right atrial cardiomyocyte of ISIAH rat on the 12th day of life (electronogram). *a*) abundant secretory granules in the cytoplasm of muscle cell; *b*) morphological types of secretory granules: *1*) forming in the Golgi zone; *2*) mature forms; *3*) dissolving forms.

was less in atrial cardiomyocytes of 12-day-old ISIAH than in age-matched control rats (Table 1). However, analysis of the morphological types of granules showed that this was due to predominance of forming and dissolved types constituting ~70%. Mature forms constituted only 30% granules (Fig. 2) and they were significantly larger than in the control animals (Table 1). These data are interpreted as the signs of active synthesis and secretion. Mature secretory granules constitute half of granular pool in age-matched control rats, which can be regarded as accumulation of synthesized peptides in the cells. In addition, the sarcoplasm in WAG rat pups contained significantly greated number of atypical granules (18 *vs.* 5 in ISIAH rats). These were large bodies, ~500 μ in diameter, ultrastructurally

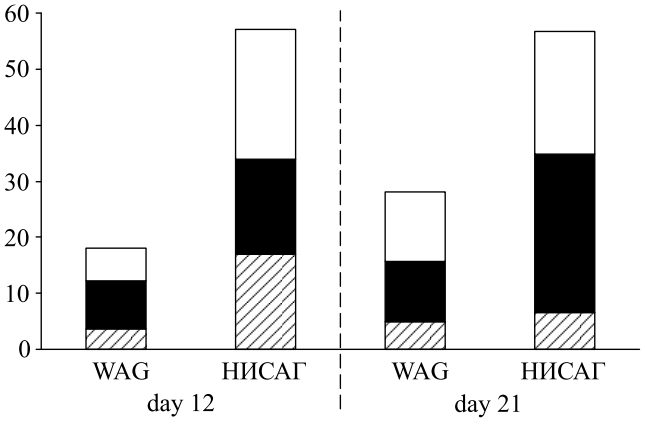

Fig. 2. Numerical density (per 100 μ²) and composition of secretory granules in the cytoplasm of atrial cardiomyocytes in 12- and 21-day-old WAG and ISIAH rats. Light bars: dissolving forms; dark bars, mature forms; hatched bars, forming granules.

similar either to very large or fused granules, granules fused with lysosomes. This was probably a sign of intracellular excess and recycling of accumulated and non-released secretion by autophagy (crinophagy).

Over the next 10 days of life, glycogen content regularly decreased, and the relative amount of contractile sarcoplasmic proteins increased in the course of cell differentiation in rats of both strains. The diameter of atrial muscle cells remained unchanged. Hence, the interstrain differences in the cell diameter; volume of Golgi complex and granules, as well as their number persisted over 3 postnatal weeks (Table 1).

The size and composition of the granules in ISIAH rats considerably changed over the next 10 days of life (from 12 to 21 day of life): they became significantly larger than in control animals due to the overwhelming predominance of mature and soluble forms. Forming granules constituted only 11% (Fig. 2). Their size did not differ from the control values and remained stable from the previous term (Table 1). These data indicate that atrial myocytes exhibit higher secretory activity in infant ISIAH rats than in age-matched controls by the end of the milk-feeding period. At the same time, changes in the ratio of synthesis, accumulation, and release of secretory granules were detected in ISIAH rats at this period of ontogeny. Peptides accumulated in granules (their size increased), the intensity of synthetic process decreased, large atypical forms with an average diameter of 547±43 μ were detected in ISIAH rats (2.5% of the total number of granules), but not in controls of this age.

Based on the physiological effects of natriuretic peptides, it is logical to assume reduced activity of this

system under conditions of hypertension and hypervolemia. In medical practice, blood levels of cardiac hormones were significantly increased in patients with cardiovascular pathology (hypertension, congestive heart failure, *etc*.) [13]. Enhanced expression of atrial natriuretic peptide was detected in spontaneously hypertensive SHR rats, in SHR rats with experimental malignant hypertension, in rats with renovascular hypertension, and with cardiac decompensation after aortocaval bypass surgery [7,12]. Researchers are trying unsuccessfully to find an explanation for the "hormonal paradox": no effect of natriuretic peptides under pathological conditions despite their abundance. Authors seek answers in inadequate compensatory responses or the failure of specific receptors in target organs [11].

These data led us to a conclusion that hormone activity of the atria in ISIAH rats is higher than in age-matched normotensive animals at least since the 12th day of life. The most striking difference was observed in early postnatal ontogeny, when the genetic pathology (hypertension) has not yet manifested. These facts cast doubts on the interpretation of cause—effect relationships during the development of genetically conditioned hypertension. It can be assumed that secretory hyperactivity of atrial myocytes does not indicate the compensatory response to changes in blood pressure and water-salt homeostasis, but indicates a new pathogenetic link, which contributes to the formation of pathology in addition to other hormonal systems.

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