PHYSIOLOGY

Structural Characteristics of Cardiomyocytes in the Right Atrium of NISAG Rats

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Electron microscopy and stereomorphometric analysis of hypertrophic cardiomyocytes in the right atrium of NISAG rats revealed signs of activation of biosynthetic processes: increased relative volume of euchromatin (compared to Wistar rats), high density of nuclear pores, presence of large numerous Golgi complexes, and well-developed endoplasmic reticulum. The numerical density of secretory granules in the cytoplasm of cardiomyocytes in NISAG rats significantly surpassed that in Wistar rats. However, these granules in NISAG rats were smaller than in Wistar rats. The presence of numerous secretory granules and increased ratio of forming and dissolving structures suggest that this pool is characterized by high turnover rate, *i.e.*, intensive synthesis and rapid elimination (consumption) of natriuretic peptide. Hypertrophy and hyperactivity of endocrine function in atrial cardiomyocytes of NISAG rats can be considered as a compensatory reaction to hypertension.

Key Words: *secretory granules; atrial cardiomyocytes; natriuretic hormone; arterial hypertension*

Rats with hereditary stress-induced arterial hypertension (NISAG) are highly sensitive to stress. The development of stable arterial hypertension in adult animals is accompanied by specific morphological changes in vitally important organs (*e.g.*, in the heart) [2,4]. Polypeptide hormones (natriuretic peptides, NP) are secreted in the atria and play a role in the regulation of blood pressure [9]. Ultrastructural histochemical and immunocytochemical studies showed that specific atrial granules contain NP prohormones [13]. NP content in the right atrium is 1.5-3 times higher than in the left atrium [7]. Since it is impossible to perform a morphological study of atrial cardiomyocytes (CM) in patients with hypertension, most modern researches are conducted on inbred animals [1]. Previous studies showed that cardiac hormones can be used for therapeutic purposes [3,5]. However, this problem requires detailed investigations.

Here we performed a qualitative and quantitative electron microscopic study of the secretory apparatus in right atrial CM of hypertensive NISAG rats.

MATERIALS AND METHODS

Experiments were performed on 6 male NISAG rats aging 6 months and weighing 292.0±8.5 g. The mean blood pressure at rest was 160.0±3.3 mm Hg. The control group included normotensive Wistar rats of the same age.

The heart was removed under ether anesthesia. For electron microscopy, the right atrial myocardium was fixed in 2.5% glutaraldehyde and 2% paraformal-

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TABLE 1. Morphometric Indexes of CM Nuclei in the Right Atrium of Wistar and NISAG Rats (*M±m*)

Rats	CM diameter, μ	Nucleus section area, μ²	Relative volume in nucleus, %		Number of pores per 10 μ membrane
			euchromatin	nucleolus	
Wistar (*n*=40)	10.2±0.2 (5.8-13.8)	13.30±0.98	74.20±0.93	2.57±0.63 (0-15.1)	4.41±0.18
NISAG (*n*=47)	11.20±0.22* (4.6-16.1)	14.20±0.84	79.60±0.92*	2.13±0.54 (0-13.6)	5.76±0.21*

Note. Range of values is shown in brackets. Here and in Table 2: *$p<0.05$ compared to Wistar rats.

dehyde, postfixed in 1.5% OsO_4, and embedded into Epon and Araldite. The diameter of CM nuclei was measured on semithin sections stained with toluidine blue. The measurements were performed using an ocular rule (increment 2.3 μ, ×700). Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined under a JEM 100SX electron microscope.

We performed a qualitative and stereomorphometric study of right atrial CM. The relative volume of the main structures in CM and number, density, and size of secretory granules were estimated with a 72-point closed square test grid at an initial magnification of 5000. The morphological study determined the composition of secretory granules. We evaluated the relative volume of euchromatin, heterochromatin, and nucleoli in CM nuclei. The number of pores per nuclear membrane perimeter was estimated.

The significance of differences between the means was evaluated by Student's *t* test and nonparametric Mann—Whitney—Wilcoxon test.

RESULTS

Light microscopy showed that the mean cross-section diameter of atrial CM at the level of their nuclei in NISAG rats was much higher and underwent more significant variations than in Wistar rats (Table 1).

Hypertrophy of CM was proportional by the ratio of organelles. The volume ratio of the main structures did not differ from the control (except for mitochondria, Table 2). No differences were found in the size of nuclei and relative volume of nucleoli in myocytes of NISAG and Wistar rats. However, in NISAG rats the content of nuclear euchromatin was higher than in Wistar rats, heterochromatin looked like a narrow peripheral band, and nucleoli often had a nucleonemal structure (Fig. 1, *a*, *b*). The density of pores in the nuclear membrane in NISAG rats was higher than in Wistar rats (Table 1).

Cytoplasmic secretory granules were predominantly localized in the paranuclear Golgi complex. In NISAG rats we often found Golgi complexes with widened cisternae that lay at the periphery of CM (as distinct from Wistar rats). Sections of some cells included 4-7 Golgi complexes surrounded by granules and large lysosomes with heterogeneous content (Fig. 1, *c*). In some CM, secretory granules were arranged in groups near the Golgi complex. The number of granules in the section reached 150-180. Other (sometimes adjacent) cells included only solitary granules. The numerical density of secretory granules in NISAG rats was higher than in Wistar rats (per 100 μ² cytoplasm). At the same time, the size of granules in NISAG rats was lower than in Wistar rats (Table 1). We revealed no differences in the total relative volume of granules in CM of NISAG and Wistar rats (Table 2). In NISAG rats many granules lay in the peripheral zone under the sarcolemma (Fig. 1, *c*). Electron microscopic signs of formation, maturation, and dissolution of secretory granules in CM were similar in NISAG and Wistar rats. However, the qualitative composition of granules differed in animals of these strains. As differentiated from Wistar rats with the prevalence of mature structures, NISAG rats had a considerable number of forming and dissolving granules (Fig. 2).

TABLE 2. Morphometric Indexes of CM in the Right Atrium of Wistar and NISAG Rats (*M±m*)

Rats	Relative volume in cytoplasm, %					Number of granules per 100 μ²	Section area for granule, μ²
	myofibrils	mitochondria	Golgi complex	secretory granules	cytoplasm		
Wistar (*n*=74)	43.20±0.63	28.60±0.56	1.93±0.21	3.79±0.26	21.90±0.49	28.00±1.56	0.122±0.050
NISAG (*n*=75)	41.7±0.5	31.70±0.41*	2.21±0.22	3.83±0.25	21.90±0.51	34.80±2.54*	0.100±0.032*

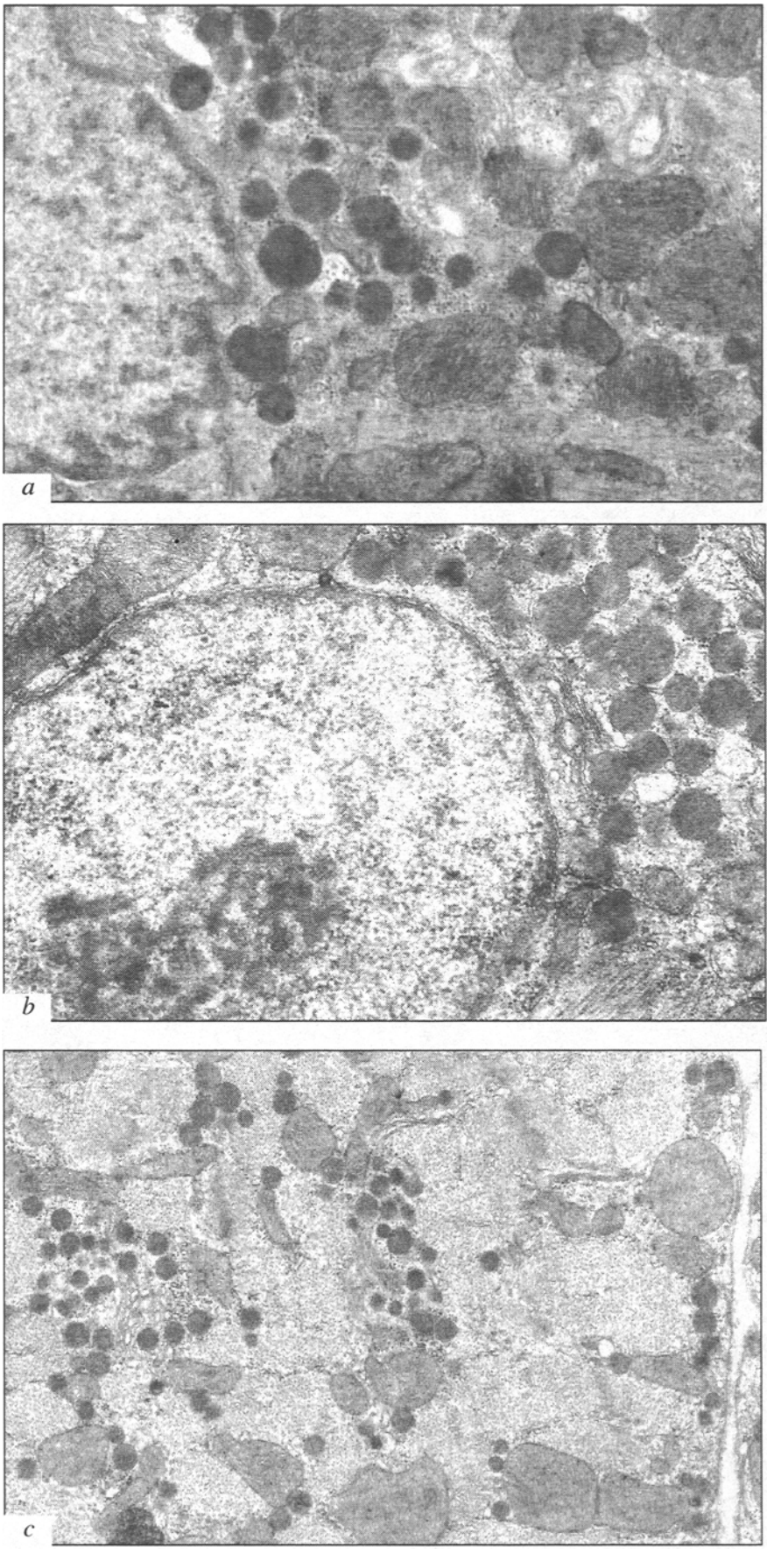

Fig. 1. Ultrastructure of cardiomyocyte in the right atrium of rats. Single large secretory granules in the paranuclear space of Wistar rat (×20,000, *a*). Nucleonemal nucleolus in the nucleus with a considerable amount of euchromatin, numerous secretory granules in the Golgi complex, and large number of dissolving granules in NISAG rat (×20,000, *b*). Numerous secretory granules in the central and peripheral region of myocytes and hyperplasia of Golgi complexes in NISAG rat (×15,000, *c*).

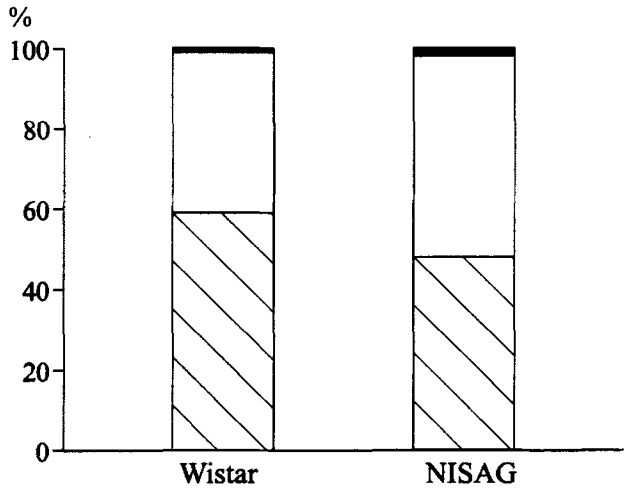

Fig. 2. Composition of secretory granules (%) in right atrial cardiomyocytes of Wistar and NISAG rats. Significant interstrain differences at $p<0.05$. Light bars: dissolving granules. Dark bars: forming granules. Shaded bars: mature granules.

In NISAG rats large CM of the right atrium had morphological signs of activation of biosynthetic processes: increased relative volume of euchromatin, high density of nuclear pores, large numerous Golgi complexes, and well-developed endoplasmic reticulum in the sarcoplasm. The presence of numerous secretory granules in the cytoplasm and increased ratio of forming and dissolving structures suggest that this pool is characterized by high turnover rate, *i.e.*, intensive synthesis and rapid elimination (consumption) of the hormone. Our assumption is confirmed by lower diameter of secretory granules in NISAG rats compared to Wistar rats (0.35 and 0.39 μ, respectively). In NISAG rats secretory granules could not reach large volume. This is a widely accepted interpretation of morphological changes [6] observed during the reduction of myocyte granulation in rats with spontaneous [15] and renovascular hypertension [8]. In atrial myocytes of hypertensive rats, the state of organelles involved in hormone synthesis and volume of mitochondria responsible for energy supply are characterized by greater functional variability than in normotensive animals. Hypertrophy and hyperactivity of endocrine function in atrial CM of NISAG rats can be considered as a compensatory reaction to hypertension.

NP not only intensifies sodium release from the organism, but also increases diuresis, causes relaxation of vascular smooth muscles, decreases blood pressure, and acts as antagonist of the renin—angiotensin—aldosterone system. Various humoral factors stimulate the release of NP from secretory granules of CM into the circulation and activate *de novo* synthesis of this peptide. Mechanical strain of atrial cells most significantly modulates this process. In rats with experimental hypertension (aortocaval bypass, renovascular hypertension, and DOCA-salt models) a strong correlation was found between intensification of NP synthesis in the myocardium, its blood content, and degree of heart hypertrophy [11,14]. These data confirm the suggestion that secretory activity of atrial CM considerably increases in NISAG rats. These rats are characterized by pronounced hypertrophy of the heart. The relative weight of the heart and the mean diameter of left ventricular myocytes in NISAG rats were 3.57± 0.10 mg/g and 21.10±0.37 μ, respectively (*vs.* 3.03± 0.07 mg/g and 14.70±0.29 μ in the control, respectively) [4]. It was hypothesized that the increased level of NP in the blood can serve as a marker of myocardial hypertrophy [12]. Atrial peptides produce a direct antihypertrophic effect in mice with genetic knockout of these hormones. Therefore, overproduction of atrial peptides plays a compensatory role in NISAG rats [10].

Our results indicate that the development of hereditary stress-induced arterial hypertension is accompanied by hypertrophy of not only left ventricular myocardium, but also right atrial CM, intensive synthesis of the hormone, and its rapid release from atrial myocytes.

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