MORPHOLOGY AND PATHOMORPHOLOGY

Atrial Cardiomyocytes of a Heterothermic Animal in Various Physiological States

M. S. Vinogradova, L. N. Cherezova, I. M. Korostyshevskaya, and L. V. Kolesnikova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 126, No. 9, pp. 353-356, September, 1998
Original article submitted December 22, 1997

Hormone-producing cardiomyocytes of the right atrium of suslik were studied in summer and winter periods (the torpidity and spontaneous awake states). The hormone was shown to be intensely synthesized in summer. The intensity of this process decreased during hypothermia and increased during winter spontaneous awakenings. The role of these changes in the maintenance of water and salt homeostasis is discussed.

Key Words: *cardiomyocytes; atrial natriuretic peptide; winter hibernation*

Cardiomyocytes (CM) of the atria perform contractile and endocrine functions and produce atrial natriuretic peptide (ANP). ANP is a polypeptide that decreases blood pressure and increases natriuresis. It is an antagonist of the renin-angiotensin-aldosterone system [2,11]. There are no data on the hormone-producing activity of atrial cells of animals that undergo hibernation in winter.

Here we studied specific morphofunctional features and secretory activity of atrial CM (ACM) of heterothermic mammals under various seasonal and physiological conditions.

MATERIAL AND METHODS

The auricles of the right atria of *Citellus erythrogenys Brandt* were studied in three physiological states: the summer activity (five animals with a body temperature of 37°C), torpidity (five animals with a body temperature of 6-8°C), and winter spontaneous awakening (four animals with a body temperature of 35-36°C).

The material was fixed in paraformaldehyde-glutaraldehyde, postfixed in osmium tetroxide, and embedded in Epon-Araldite. Ultrathin sections were contrasted according to the Reynolds procedure and studied in a Hitachi-600 electron microscope. Semithin cross-sections were stained with azure. The diameters of ACM and their nuclei were measured. Morphometrical parameters of ultrastructures were obtained from negatives at a final magnification of 40,000. Results were analyzed statistically using the STATGRAPHICS software and Student's *t* test.

RESULTS

In summer, the structure of ACM of *Citellus erythrogenys Brandt* did not differ from that typical of other mammals (Fig. 1). The composition of the nucleus and cytoplasmic organelles confirmed the activity of their hormone-producing function (Fig. 2, *a*). The produced substance was deposited in secretory granules presumably located near the nucleus. Electron-dense granules with adjacent mem-

Institute of Regional Pathology and Pathomorphology, Siberian Division of the Russian Academy of Medical Sciences; Novosibirsk State University; Institute of Physiology, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk; "Vektor" State Scientific Center for Virology and Biotechnology, Kol'tsovo, Novosibirsk region

0007-4888/98/0009-0957$20.00

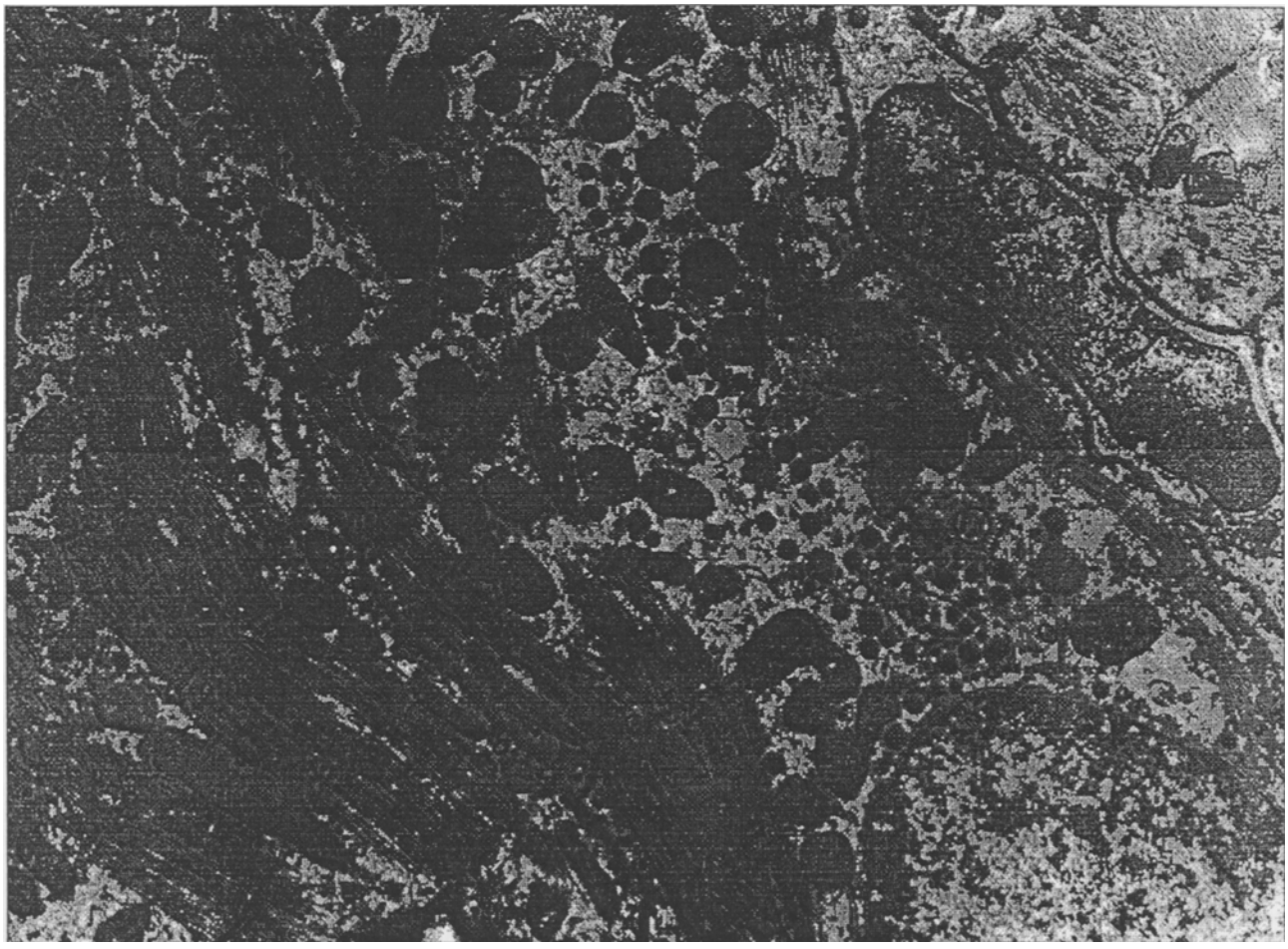

Fig. 1. Fragment of the right atrium auricle cardiomyocyte of suslik in summer. ×12,500.

branes (mature granules) and granules with a halo, a narrow light space surrounding the membrane (immature granules), were the prevalent type of secretory granules [13]. Fused granules were fuzzy and had no membranes. These findings suggest that intense synthesis, considerable accumulation, and moderate secretion of ANP occur in ACM of susliks in summer.

The susliks were in the state of torpidity in December and January. Their body temperature decreased to 2-5°C. There was a significant decrease (by 35%) in the mean diameter of ACM (Table 1). Under similar conditions the diameter of CM of the left ventricle decreased only by 16% [8]. This was probably due to the necessity to maintain pump function of the heart and hemodynamic parameters in the state of torpidity (the blood pressure of 50/30 mm Hg and the pulse rate of about 10 beats/min) [19]. The mean diameter of ACM nuclei and the relative volume of euchromatin decreased. Therefore, their functional activities also decreased [5]. Under these conditions nucleoli with a nearly undetermined structure were observed more rarely than in summer. Ribosomes bound to the individual cisternae of the rough endoplasmic reticulum (RER) discharged their contents. The cytoplasm was enriched with free ribosomes and glycogen granules.

The Golgi apparatus (GA) consisted of small associations of vesicles of various diameters and lacked flat cisternae (Fig. 2, *b*). All morphometric indices of the GA decreased significantly. Similar changes in the GA were observed in the heart ventricle myocytes of susliks in winter [10]. All numerical parameters of secretory granules decreased significantly. Mature granules were small. Fused granules with moderately dense cores and hardly determined (or undetermined) membranes were ubiquitous. Immature granules were not found. Electron-dense bodies of calcium phosphate (probably), which is deposited with the decrease in the RER activity, appeared in the mitochondrial matrix [7]. The content of lipid droplets increased. Similar changes were observed in CM of the heart ventricles [4]. These data suggest that a considerable decrease

in the synthesizing activity of ACM accompanied by consumption of the earlier produced secretory product occurred during torpidity.

In susliks awaked spontaneously in winter (whose body temperatures approached normal levels), the diameters of cells and nuclei increased significantly (by 15% and 16%, respectively) compared with those in animals in the state of hibernation. However, these parameters did not reach the values described for susliks that were active in summer. The content of euchromatin in the nuclei was 9.5% higher than that in animals in deep hibernation and only 5% lower than that in summer. The nucleoli were often observed in the section. Their relative volume was 37% higher than that in animals in deep hibernation and 29% lower than that in summer. Flat cisternae appeared in the GA (Fig. 2, *c*). In some cases, formation of granules was observed. The relative volume of the GA and surface density increased by 7% and 37% (respectively) in the state of spontaneous awakening as compared to these parameters in deep hibernation.

The relative volume and surface density of the GA in spontaneously awaked susliks were lower by 39% and 42% (respectively) in comparison with these parameters in susliks observed in summer. The numerical density of secretory granules tended to increase by 16%, and their mean diameter increased significantly by 17% in comparison with these parameters in susliks in the state of torpidity, and were lower by 64% and 17%, respectively, than those in the summer period. Electron-dense granules with a halo (immature) and fuzzy granules of moderate density and vague membranes (or lacking them) prevailed. Mature granules were small. Electron-dense bodies disappeared from the mitochondrial matrix. Lipid droplets were less abundant than in the hibernation state. They were small.

These data indicate that spontaneous awakening is accompanied by activation of the synthesizing system of ACM. Synthesis and secretion of the hormone are the dominant processes. The intensity of its deposition is low. All morphometric indices of ACM studied in this work differed significantly from those described for animals in the state of summer activity. However, comparison of these parameters in animals in deep hibernation and spontaneous awakening states showed that not all the differences were statistically significant (Table 1). The cells probably do not reach high level of metabolic activity over short periods of spontaneous awakenings in winter.

ANP secretion is a special mechanism of regulation of blood pressure and water and salt homeostasis in the body [1]. Obviously, this mechanism operates during spontaneous awakenings of susliks.

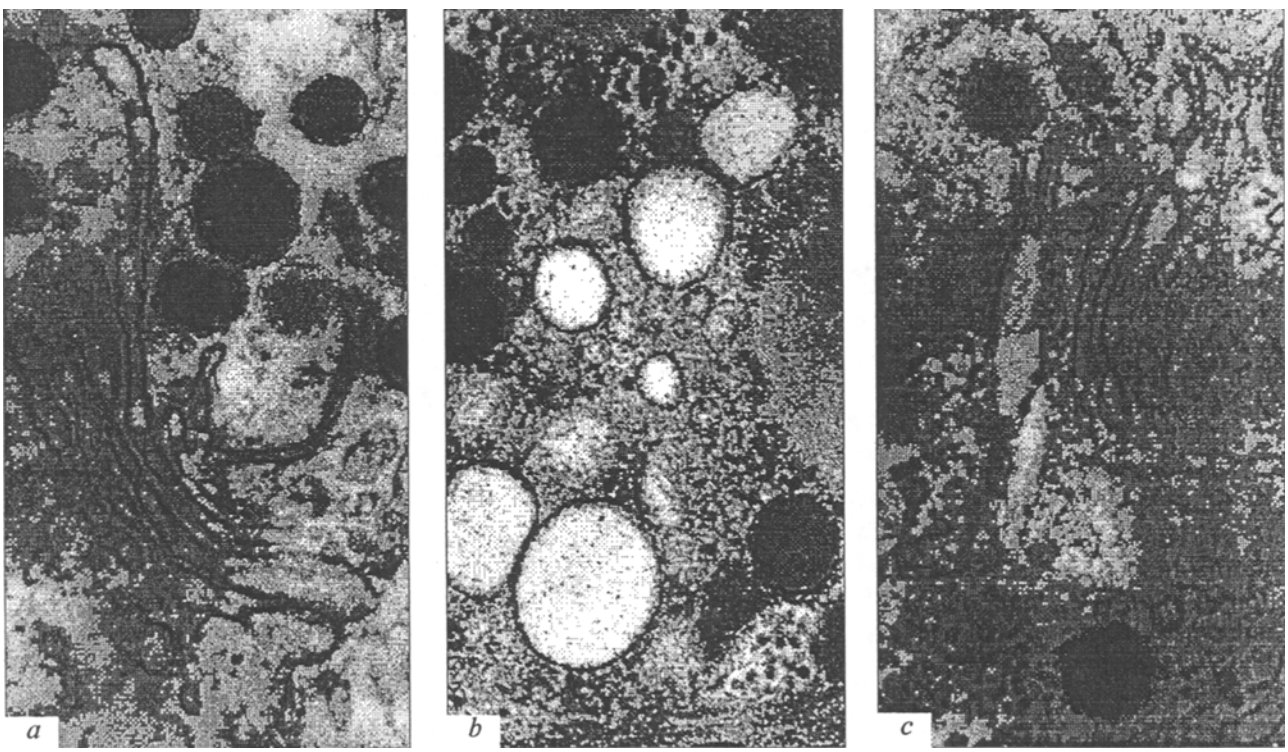

Fig. 2. Golgi apparatus of atrial cardiomyocytes of suslik in various physiological states: *a*) summer activity; *b*) winter torpidity; and *c*) spontaneous awakening in winter. ×60,000.

TABLE 1. Morphometrical Indices of ACM of *Citellus erythrogenys Brandt* in Various Physiological States (*M±m*)

Index	Summer activity	Winter hibernation	Spontaneous awakening
Diameter of CM, mm	13.5±0.22	8.8±0.07*	10.1±0.12*+
Diameter of the nucleus, mm	5.3±0.08	3.8±0.05*	4.2±0.07
Relative volume of the nucleolus, %	8.3±0.32	4.9±0.23	4.3±0.23
Relative volume of euchromatin, %	86.0±0.47	74.3±0.40*	81.4±0.58*+
Relative volume of the GA, %	7.2±0.25	4.1±0.19*	4.4±0.22*
Surface density of the GA, mm^{-1}	1.9±0.5	0.8±0.03*	1.1±0.04*+
Relative volume of granules, %	13.1±0.64	4.0±0.24*	5.1±0.31*+
Numerical density of profiles of granules, mm^2	18.0±0.82	5.5±0.37*	6.4±0.46*
Surface density of granules, mm^{-1}	2.0±0.09	0.6±0.005*	0.7±0.05*
Diameter of granules, mm	0.24±0.005	0.17±0.004*	0.20±0.006*+

Note. $p<0.05$: *compared with the winter period; +compared with the period of torpidity.

First, heart temperature in hibernators increases [3,12]. When the body temperature of susliks is 10-15°C, the heart rate is already 160-250 beats/min [9]. High heart rate, increased blood pressure, and the load on the atrium stimulate the secretion of ANP, which provides the regulation of the heart and vascular system and kidneys. The kidneys of susliks do not produce urea during the state of torpidity [6,12]. From September to April these animals do not eat and drink. Their exogenous equilibrium of water and electrolytes changes significantly. Cells of their organs are dehydrated and accumulate excessive amounts of cations. Their function is restored (for a short period of time) during awakenings. ANP is probably involved in this process.

Thus, the morphology of ACM of *Citellus erythrogenys Brandt* changes significantly during the hibernation-activity cycle. ANP is produced and secreted by CM of heterothermic animals according to the physiological state of the body. This hormone is obviously involved in the regulation of water and electrolyte equilibrium in the body of hibernators (particularly, in spontaneous awakenings during winter hibernation).

REFERENCES

1. D. Kh. Akramova and I. A. Chervova, *Arkh. Anat.*, No. 8, 5-14 (1989).
2. N. A. Artemyan and I. D. Shperling, *Byull. Eksp. Biol. Med.*, **101**, No. 6, 760-766 (1986).
3. G. F. Zhegunov, *Zhurn. Evoluts. Biokhim.*, No. 5, 647-651 (1987).
4. G. F. Zhegunov and E. V. Kudokotseva, *Kriobiologiya*, No. 1, 36-39 (1989).
5. I. B. Zbarskii, *Organization of the Cell Nucleus* [in Russian], Moscow (1988).
6. L. N. Ivanova, in: *Ecological Physiology of Animals. Part I.* [in Russian], Leningrad, 165-178 (1979).
7. E. Karafoli and J. T. Penniston, *V Mire Nauki*, No. 1, 28-38 (1986).
8. L. V. Kolesnikova and L. M. Nepomnyashchikh, *Arkh. Anat.*, No. 4, 52-59 (1984).
9. I. M. Korostyshevskaya, V. F. Maksimov, and A. V. Mizerov, in: *Ecological and Physiological Characteristics of Natural Hypometabolic States* [in Russian], 2, 3-8, Novosibirsk (1992).
10. L. M. Nepomnyashchikh, L. V. Kolesnikova, and G. I. Nepomnyashchikh, *The Morphology of Cardiac Atrophy: Three-Chamber Tissue and Ultrastructural Organization* [in Russian], Novosibirsk (1989).
11. A. I. De Bold, H. G. Borenstein, A. T. Veress, and H. Sonnenberg, *Life Sci.*, **28**, 89-94 (1981).
12. Ch. Kayser, *The Physiology of Natural Hibernation*, New York (1961).
13. J. N. Skepper and V. Navaratham, *Histochem. J.*, **20**, 1-10 (1988).