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MORPHOLOGICAL FEATURES OF NATRIURETIC FACTOR SECRETION BY ATRIAL CARDIOMYOCYTES IN SPONTANEOUSLY HYPERTENSIVE RATS

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In 1956, Kisch [4], in an electron-microscopic study of guinea pig cardiomyocytes, found small granules which were present only in the atrial cardiomyocytes (ACM). In a more detailed study of the ACM and the system forming specific granules, Jamieson and Palade [3] expressed, for the first time, the view that ACM possess a secretory as well as a contractile function. De Bold et al. [1, 2] proved that the atrial granules contain a substance which has a powerful natriuretic and diuretic action, which they called atrial natriuretic factor (ANF). Subsequent investigations showed that ANF is a short polypeptide, which not only possesses natriuretic and diuretic activity, but also has a relaxing action on smooth-muscle cells and can inhibit the renin–angiotensin–aldosterone system [6]. It has been suggested that ANF plays an important role in the genesis of arterial hypertension.

The aim of this investigation was a morphological assessment of the system synthesizing and secreting ANF in the cardiomyocytes of the right atrium in rats with experimental hypertension. For this purpose, the number, size, and distribution of specific granules were studied by electron microscopy and the rough endoplasmic reticulum and Golgi lamellar complex in the ACM were studied in spontaneously hypertensive (SHR) and normotensive (WKY) rats of the control group.

EXPERIMENTAL METHOD

The experimental material consisted of the auricles of the right atrium of SHR (spontaneously hypertensive Kyoto-Wistar) and WKY (normotensive Kyoto-Wistar) rats of two age groups (Table 1). The animals were decapitated under ether anesthesia in the morning, material was immersed in a 2.5% solution of glutaraldehyde in phosphate buffer (pH 7.4), after which it was postfixed with osmium and embedded in a mixture of Epon and Araldite. Ultrathin sections were cut on the LKB-III Ultrotome. The sections were stained with uranyl acetate and lead citrate [5, 7] and examined and photographed in the Hitachi IIE electron microscope (Japan) under standard magnification on the photographic plate of 6100; electron micrographs were printed under a standard magnification of twice (final magnification 12,200). In each case all cardiomyocytes having a nucleus regardless of in which plane the section was cut, and in no fewer than three ultrathin sections repaired from different areas of the auricle, were photographed. The panoramas of the cells were subjected to statistical analysis by means of the MOP-Videoplan computerized morphometric system, using standard statistical programs from Kontron (France).

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TABLE 1. Characteristics of Animals and Morphometric Data (M ± σ)

Strain of rats	Age, weeks	Body weight, g	Weight of heart, mg	BP, mm Hg	No. of cells investigated	Area of cytoplasm, μ^2	Number of granules	Diameter of granules, μ	Area of cytoplasm (in μ^2) per granule
SHR (n=4)	8	167±9,4	0,7±0,01	181,3±10,3	58	150±74	46,29±31,05**	2,17±0,63	4,57±3,15*4
WKY (n=5)	8	195±8,3	0,67±0,02	112,5±13,2	82	136±49	31,18±20,62**	2,11±0,58	7,39±6,89*4
SHR (n=3)	23	321,3±12,1	1,4±0,52	205±30,4	65	194,5±137*	77,08±61,11***	2±0,64	3,26±1,79
WKY (n=3)	23	284±7,6	0,83±0,03	136,7±10,4	55	133±74*	52,64±40,22***	2,14±0,67	3,58±2,34

Legend. *) Differences in area of cytoplasm significant at $p < 0.01$ level between SHR and WKY aged 23 weeks, **) differences in number of granules in cell significant at $p < 0.01$ between SHR and WKY aged 8 weeks, ***) differences in number of granules in cell are significant at $p < 0.05$ between SHR and WKY rats aged 23 weeks, ****) differences in area of cytoplasm per granule significant at $p < 0.01$ level between SHR and WKY rats aged 8 weeks. n) Number of rats.

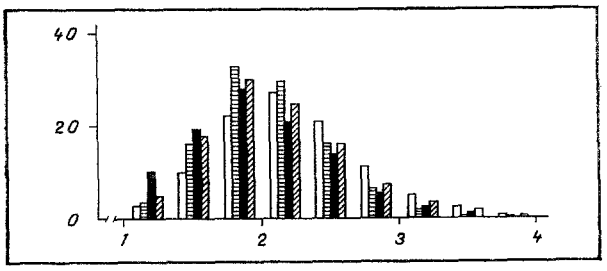

Fig. 1. Distribution of atrial granules among classes depending on their maximal diameter. Abscissa, maximal diameter (in μ); ordinate, distribution (in %). Unshaded columns — SHR aged 8 weeks, horizontally shaded — normotensive rats of the same age; black columns — SHR aged 23 weeks, obliquely shaded — normotensive rats of the same age.

EXPERIMENTAL RESULTS

The granules were small, round bodies about 2 μ in diameter, surrounded by a single layer of membrane. Their matrix was dense and homogeneous. The granules were mainly distributed near the poles of the nucleus, but single granules were found among the myofibrils and near the cytoplasmic membrane. Outflow of the granules or their contents from the cell was not observed. The increase in the number of granules in SHR was noted, and was confirmed by the morphometric data (Table 1). With age the number of granules increased in animals of both groups. Hypertrophy of the ACM was observed in the SHR. The mean diameter of the atrial granules in rats of both groups only just exceeded 2 μ (Table 1). A significant decrease in diameter of the atrial granules was observed in SHR at the age of 23 weeks, due to an increase in the number of granules under 2 μ in diameter (Fig. 1). Elements of a Golgi complex and the rough endoplasmic reticulum also were found more frequently in SHR. Elements of the lamellar complex were found in some cardiomyocytes of the rats of this group not only in the perinuclear zone, but also freely scattered in the cytoplasm.

The main result of this investigation is the conclusion that the system responsible for production of ANF in SHR has distinct features of activation of its synthetic and secretory functions and is hypertrophied. This is shown both by the increase in the number of atrial granules (by almost 1.5 times in both age groups) and by hypertrophy of the lamellar Golgi complex and rough endoplasmic reticulum in ACM. This conclusion, at first glance, contradicts the data of Sonnenberg et al. [8], who found that the diuretic activity of atrial extract of SHR was weaker than that of normotensive rats. However, this contradiction disappears if the differences in the methods are analyzed. In the investigation cited activity was calculated per gram weight of atrium, whereas we calculated it per cell. Because of hypertrophy of the cardiomyocytes (Table 1), the ratio of the area of the cytoplasm to the number of granules, i.e., the area of the cytoplasm (and also its weight) per granule, at the age of 23 weeks was about equal both in the experiment and in the control. It must also

be recalled that the granules contain mainly a precursor protein which has evidently less activity than the active ANF peptides. It is natural to suggest that with an increase in the demand for ANF (and an increase in its utilization) the granules will contain less of the active peptides, or the concentration of the material of the granules will be smaller. Thus the contradiction between our results and those of Sonnenberg et al. [8] is easily explained. When the role of increased production of ANF in the pathogenesis of essential hypertension is examined, it must be noted that this factor, in its physiological action, is of course one of the regulators of the blood pressure (BP). It is natural to suggest that when BP is raised, to protect homeostasis the production of a natural "hypotensive agent" will be intensified. There is another possible explanation: the ANF system, which regulates the excretory function of the kidneys, reacts on the new conditions of work at an increased pressure so as to maintain the state of "switching" of the kidney characteristic of chronic hypertension. Finally, the possibility cannot be ruled out that stimulation of ANS secretion reflects the existence of a genetic defect in the excretory function of the kidney and is aimed at its correction. Further research will shed light on the pathophysiological role of the changes discovered in the ANF producing system in spontaneously hypertensive rats.

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