Atrial Natriuretic Peptide (ANP)-Granules of Auricular Cardiocytes in Aging Mongolian Gerbils

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The atrial natriuretic peptide (ANP)-granules of right auricular cardiocytes were studied in aging Mongolian gerbils by immunohistochemistry and transmission electron microscopy, and analyzed by ultrastructural morphometry. Immunohistochemically, the ANP immunoreactions were weaker in 3 and 4-year-old animals than in 90-day-old, 1- and 2-year-old animals. There was no difference in the reaction among 90-day-old, 1 and 2-year-old animals, or between 3 and 4-year-old animals. Ultrastructurally, the morphological features in 1 and 2-year-old animals were similar to those of 90-day-old animals, but distinct in 3 and 4-year-old animals by the presence of a few lysosomal structures in the cell. By ultrastructural morphometry, number and diameter of ANP-granules of 3 and 4-year-old animals were significantly smaller than those in the 90-day-old, 1 and 2-year-old. There was no significant difference in number and diameter among the 90-day-old, 1 and 2-year-old, or between the 3 and 4-year-old.

Key Words: aging, atrial natriuretic peptide, cardiocyte, Mongolian gerbil

It is well known that the atrial cardiocytes in mammals comprise endocrine cells which secrete atrial natriuretic peptide (ANP) with diuretic, natriuretic and vasodilatory properties and inhibitory action of aldosterone, cortisol, arginine vasopressin and renin release. In our previous report, the morphological character of ANP-granules in cardiocytes of adult Mongolian gerbils was clarified. However, their morphological changes with aging have not yet been reported. Thus, this paper describes structural and distributional changes of the ANP-granules during aging in Mongolian gerbils by immunohistochemistry and transmission electron microscopy. The differences in number and diameter of the ANP-granules were also analyzed by morphometry in the right auricular cardiocytes of these animals.

The hearts were obtained from 5 males in each group of 90-day-old, 1, 2, 3, and 4-year-old Mongolian gerbils and used in this study. All animals were kept in automatically controlled rooms (temperature: 24 ± 1°C; humidity: 55 ± 5%; automatic lighting: 6:00 a.m. to 8:00 p.m.) and fed a pellet diet CE-2 (CLEA Japan, Inc. Osaka, Japan) and water ad libitum. Auricular tissues were removed from these animals under Nembutal anesthesia. For immunohistochemical examinations, right auricular tissue blocks were fixed with Zamboni's solution for 24 hr at room temperature. Immunohistochemical staining was performed according to the modified...
avidin–biotin–peroxidase complex (ABC) technique as follows. The deparaffinized sections were incubated with primary antibody overnight at 4 °C. The antibody was raised in the rabbit against atrial natriuretic peptide (human, 1-28) [Peptide Labs, Japan] and diluted 1: 500 for use with PBS containing 0.02% Triton X 100. Further incubation was performed with biotinylated swine anti-rabbit immunoglobulin (1: 500; DAKO, Denmark) and then with avidin–biotin–peroxidase complex (DAKO, Denmark). Staining for peroxidase was performed using 4% diaminobenzidine and 0.004% H2O2 in Tris-HCl buffer (pH 7.6). For electron microscopic examination, the tissue blocks of the right auricle were fixed in 2% paraformaldehyde–2.5% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.4 and post-fixed in 1% osmium tetroxide in the same buffer. The specimens were dehydrated in a graded series of ethanol and embedded in Epon 812. Thin sections were double-stained with uranyl acetate and lead citrate and examined with a JEM-2000 EX electron microscope. For ultrastructural morphometry, the number and diameter of ANP-granules in right auricular cardiocytes were measured according to our previous methods [10, 11]. In each age group of gerbils, the means (± SD) of counts were calculated in 50 photographs from 5 animals with the measurements of at least 300 granules in total and statistically analyzed by Student's t test.

By immunohistochemistry, ANP immunoreactivity was detected in the cytoplasm of the cardiocytes of the right auricle in all groups examined. The ANP reaction deposits were strongly demonstrated in the perinuclear regions of the cardiocytes (Fig. 1). There was no difference in the reaction among 90-day-old, 1 and 2-year-old animals (Figs. 1a, b). The reaction was weaker in 3 and 4-year-old animals than in 90-day-old, 1 and 2-year-old (Figs. 1a, b). In 3 and 4-year-old animals, however, a few lysosomal structures were observed in the cell, and increased with age (Figs. 1c, d).

Golgi apparatus (Fig. 2a). A few granules were observed throughout the sarcoplasmic layers intervening between myofibrilar bundles. There were no different morphological features among 90-day-old, 1 and 2-year-old animals (Figs. 2a, b). In 3 and 4-year-old animals, however, a few lysosomal structures were observed in the cell, and increased with age (Figs. 2c, d).

By ultrastructural morphometry, number and diameter of the granules of right auricular cardiocytes were analyzed as shown in Table 1. In the auricular cardiocytes, the number of ANP-granules was significantly less in 3 and 4-year-old animals than in 90-day-old, 1 and 2-year-old animals (p<0.01). The diameter of the granules in the 3 and 4-year-old was significantly smaller than that in the 90-day-old, 1 and 2-year-old (p<0.01). There was no significant difference in the number and diameter among the 90-day-old, 1 and 2-year-old, or between the 3 and 4-year-old.

It is well known that the difference in size and number of ANP-granules in the cardiocytes is caused by the functional changes in ANP circulation [3, 7]. As a rule, the granulation in the cardiocytes increases due to volumeloading by increasing water sodium intake in rats, and seems to be caused by increased synthetic and secretory activity in ANP circulation [6]. Further, in other endocrine organs, hormonal ability is generally reduced with age [1, 4, 14, 18], especially in the case of aldosterone [6] and renin [12]. Since it is well-established that ANP inhibits aldosterone and renin release [9], less granules in aged animal in this study are possibly interpreted as the results of the decreased synthetic and secretory ability of ANP in the cardiocytes, or decreased ANP circulation with age. This may attribute

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**Table 1. Number and diameter of ANP-granules in the right auricular cardiocytes in aging Mongolian gerbil (means±SD)**

<table>
<thead>
<tr>
<th>Age</th>
<th>Number</th>
<th>Diameter (nm)</th>
</tr>
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<tbody>
<tr>
<td>90day</td>
<td>66.5±9.6</td>
<td>282.5±58.9</td>
</tr>
<tr>
<td>1yr</td>
<td>65.4±9.1</td>
<td>278.1±28.5</td>
</tr>
<tr>
<td>2yr</td>
<td>62.2±8.9</td>
<td>283.8±34.6</td>
</tr>
<tr>
<td>3yr</td>
<td>43.7±12.4*</td>
<td>242.1±31.7*</td>
</tr>
<tr>
<td>4yr</td>
<td>41.9±12.9*</td>
<td>235.8±28.0*</td>
</tr>
</tbody>
</table>

* significantly different from 90-day-old, 1-year-old and 2-year-old animals (p<0.01)
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the appearance of lysosomal structures in the aged cardiocytes to be caused by reduced ANP circulation due to decreased systematic metabolism in aged animals. The appearance of lysosome with age is also reported in the pituitary gland [8]. Therefore, myoendocrine cells are also suggested to reduce functions of ANP synthesis and secretion with age.

The size of ANP-granules became significantly smaller with age. It varies according to different physiological states and is small in the presence of water depletion, but is not significantly different between volume-loading and control animals [7]. In biochemical findings, mRNA of atrial ANP decreases in the dehydrated state, but increases in the volume-loaded state [15, 17]. ANP-granules may possibly become smaller due to the decrease in synthetic ability with age, although they become smaller during their rapid synthesis and secretion especially in spontaneously hypertensive rats [16]. In cells synthesizing other peptide hormones, smaller granules are generally considered to contain a large amount of precursors and be more immature [2, 13]. If this holds true in ANP-granules, smaller granules in aged animals may suggest the increase of immature granules. At present, however, it remains unclear whether such reduction in the granule size is caused by decreased ANP synthesis or rapidly enhanced synthesis in the cell.

References

スナネズミの心耳筋細胞における心房性ナトリウム利尿ペプチド (ANP) 顆粒の加齢に伴う変化

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各齢のスナネズミにおける右側の心耳筋細胞の心房性ナトリウム利尿ペプチド (ANP) 顆粒を免疫組織化学的ならびに透過電子顕微鏡に観察し、さらにこれらのANP 頭粒の加齢に伴う変化を形態計測により解析した。免疫組織化学的に、3 および 4 年齢におけるANP 免疫反応は90日齢、1 および 2 年齢にくらべ弱かった。90日齢、1 および 2 年齢間、あるいは 3 および 4 年齢間には、反応差はなかった。電顕的に、1 および 2 年齢の形態的特徴は90日齢に類似していたが、3 および 4 年齢において細胞内にライソーム様構造物が観察された。形態計測的に、3 および 4 年齢のANP 顆粒の数と大きさは、90日齢、1 および 2 年齢にくらべ有意に少なく、小さかった。90日齢、1 および 2 年齢間、あるいは 3 および 4 年齢間の数および大きさに有意な差はなかった。

Explanation of Figures

Fig. 1. Immunohistochemical staining of ANP in right auricular cardiocytes. All magnifications are ×314. ANP immunoreaction is located primarily in the perinuclear region (arrowheads). The reaction is stronger in 90-day-old (a) and 2-year-old (b) animals than in 3 (c) and 4-year-old (d) animals.

Fig. 2. Electron micrographs of right auricular cardiocytes. Each bar represents 1 μm. The auricular cardiocyte contains a centrally located nucleus, numerous mitochondria, myofibrils, a few amounts of rough endoplasmic reticulum, well-developed Golgi apparatus and electron-dense granules. There are no different morphological features between 90-day-old (a), 1 or 2-year-old (b) animals, but a few lysosomal strutures (arrowheads) are observed in the cell in 3 (c) and 4-year-old (d) animals.