Morphometric study of age-related changes in the human intracardiac ganglia

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Key words: *human heart, ganglia, neurons, morphometry, age.*

Summary. *The present study was performed in order to determine morphological age-related changes of the human intracardiac ganglia. Paraffin sections of 40 ganglia from infants, adult and aged human hearts were stained with Picro-Mallory method.*

The ganglia area, nerve cell (with clearly visible nucleolus) area, neuron soma long axis length, perimeter, area of neuronal nuclei and neuron soma form factor were measured with the aid of computer images analyzing program "Sigma Scan Pro 5.0". Also, the neuronal density and the area occupied by nerve cells per ganglion section were calculated. The relative frequency of satellite cells, in close contact with nerve cell soma, was estimated.

Based on the data of this study, we concluded that the area of ganglia, neurons and their nuclei increased with age. Neuronal packing density significantly decreased, but the area occupied by nerve cells within the ganglia decreased non-significantly. Satellite cells were more numerous nearby ganglion neurons from infant hearts. Shape factor of neurons was stable between the groups.

In conclusion, the present study confirms significant differences in the morphology of the intrinsic cardiac ganglia with age.

Introduction

Autonomic control of cardiac function is fundamental for maintaining circulatory homeostasis under changing environmental and behavioral conditions (1). Intrinsic cardiac nervous system contains a variety of neurons scattered or packed within ganglia. For many years it was thought that cardiac ganglia contained only parasympathetic neurons, and served as simple impulse transmission stations. Recent physiological experiments indicate that the mammalian intrinsic cardiac nervous system possesses a heterogeneous population of neurons made up of afferent (2, 3), efferent (4) and interconnecting local circuit neurons (2, 5). Evidence also indicates that the processing of sensory and motor information occurs entirely within cardiac ganglia (6). Neurons are of different types: unipolar, bipolar, and multipolar (7–10) and possess different neurochemical profiles (11). Cardiac ganglia are interconnected via plexuses of nerves, widely distributed throughout the atria (12, 13). Groups of ganglia are regularly found in certain locations and constitute ganglion fields interconnected with nerve fibers of different diameter (8, 12, 14–16). Postganglionated intrinsic nerves from different ganglion fields go to predestined areas and control specific cardiac regions (1, 17). Neurons are associated with complex synapses (8, 18) and help to maintain adequate cardiac output: the power, the rate of contraction and the volume of blood delivered to cardiac muscle thorough the coronary vasculatures.

The structural modifications of the autonomic nervous system occur at different periods during development and maturity without any obvious age-stage at which neurodegenerative changes come to predominate (19). Previous morphological studies of autonomic ganglia and neurons in different species of mammals showed that they undergo morphological changes with age: loss of neurons (20–23), gain of fibrous tissue (24) and increase in neuronal size (21, 24–26). Morphometric measurements on rat sympathetic neurons of the coeliac-superior mesenteric ganglion elucidate a marked decrease in neuronal packing density with age, which was accompanied by increases in size of the neurons and their nuclei (27). The spatial density of myenteric neurons decreases dramatically in aging guinea pigs, and the total number of myenteric neurons in the small intestine was only 40–60% of the value obtained in children (21). In ageing mice trachea gang-

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lionated plexus the average number of ganglion neurons was the same as in young and old animals; however, cell sizes were markedly increased (22). In parallel to an increase in the average diameters, the number of small neurons decreased and the percentage of large neurons increased in postnatal ontogenesis of kittens stellate ganglion (26). In the nerve plexus of human esophagus (28) as well as in the small intestine (20) a decreased number of neurons is accompanied by increase in their size.

Within rat intrinsic cardiac ganglia loss of neurons and increase in connective tissue was accompanied by increase in size of remaining neurons (29). A significant reduction in the number of neurons was noted with age and it was suggested to contribute to the reduced chronotropic responses in the aged (30). Other age-related changes in the human cardiac nerve plexus include decreased activity of acetylcholinesterase and the content of catecholamines in addition to a decreased density of the plexus itself (31). The cholinergic innervation reaches full differentiation by the age of 30, and its involution begins after 50 (32). The adrenergic innervation in the intrinsic heart plexuses of the human heart is most manifested in children; its involution begins from the age of 30 in healthy persons and is at 60 years of age devoid of catecholamines (32).

Despite of all these studies, detailed data about morphological age-related changes in human intrinsic cardiac ganglia is still lacking. The aim of this work was to investigate human cardiac ganglia and ganglionic neurons morphometrically and to estimate their aging changes.

Material and methods

Hearts devoid of cardiac pathology from 11 humans of either sex were used in this study. The hearts were grouped according to age into 3 groups: infant hearts (from 9 days till 4 months old), n=4 (weighted 20–50 g); adult hearts (31–44 years old), n=4 (weighted 340–380 g) and old hearts (61–78 years old), n=3 (weighted 450–630 g). The collection of the hearts followed the ethical requirements of the institution from which they were obtained. Forty ganglia were collected from the epicardial ganglionated plexus, situated on the dorsal wall of the left atrium. The removed posterior wall of left atria was divided into 10–15 pieces and fixed in 10% formalin in phosphate buffer (pH 7.3) for 24 hours, dehydrated in an increasing alcohol series, immersed in benzene and embedded in paraffin. Transverse serial 4 mm sections were stained using Picro-Mallory method (33).

Each fifth histological profile in a row from the serial sections of the ganglia was photographed with a digital camera Olympus DP 11 and microscope Olympus BX 51. The ganglion area (on the largest section), long axis length, perimeter and shape factor (defined as $4\pi \times$ area/perimeter; the form factor equals 1 in a perfect circle) of neurons as well as area and long axis length of the neuronal nucleus were measured on nucleated neuronal profiles with visible nucleolus by a morphometric image analyzing computer program ("Sigma Scan Pro 5.0"). Neuronal packing density was calculated: number of neurons was counted on the section and this number was divided by the section area and expressed as number of neurons for 1 mm^2. Number of satellite cells surrounding each neuron soma was counted and frequency of satellite cells was expressed as one satellite cell nucleus, repeating every relative value of neuronal perimeter length. Percentage area occupied by nerve cells was calculated; sectional area of the ganglion was divided from total area occupied by nerve cells within the same ganglion section.

Data are presented as mean ± standard error of the mean (SEM). Statistical evaluations of differences were performed by one-way analysis of variance (ANOVA), followed by a post Hoc test. Significance was accepted at $p<0.005$.

Results

In all age groups the majority of ganglia were of regular round or oblong shape. Presence of connective tissue capsule around ganglion varied between groups. In the adult and ageing groups ¾ of ganglia were surrounded by thick and well-defined capsule (Fig.1). Nerve cells inside the ganglia had round or ovoid shape; they contained eccentrically located large clear nuclei and round densely stained nucleolus, usually one. Occasionally two nucleoli per nucleus were observed in the infant group (Fig. 2). Nucleoli of the intrinsic cardiac neurons were often located in the periphery inside of nerve cell nucleus (Fig. 3).

The average nerve cell soma area was 499±12 μm^2 in infant group, 738±34 μm^2 in adult and 872±32 μm^2 in aging individuals (Fig. 4A). Neuronal long axis length was 25.7±0.5 μm in infants, 33.2±0.8 μm in adults and 38.6±0.8 μm in old individuals (Fig. 4B), the difference between the groups was statistically significant ($p<0.05$). Shape factor in the infant group was 0.814± 0.004, in the adult one 0.835±0.005 and in the aged one 0.831±0.005. Nerve cell nucleus average area in infant group was 76±2 μm^2, in adult 98±4 μm^2 and in old 134±3 μm^2. The differences between infant and both other groups: adult and aged human were statistically significant (Fig. 4C).

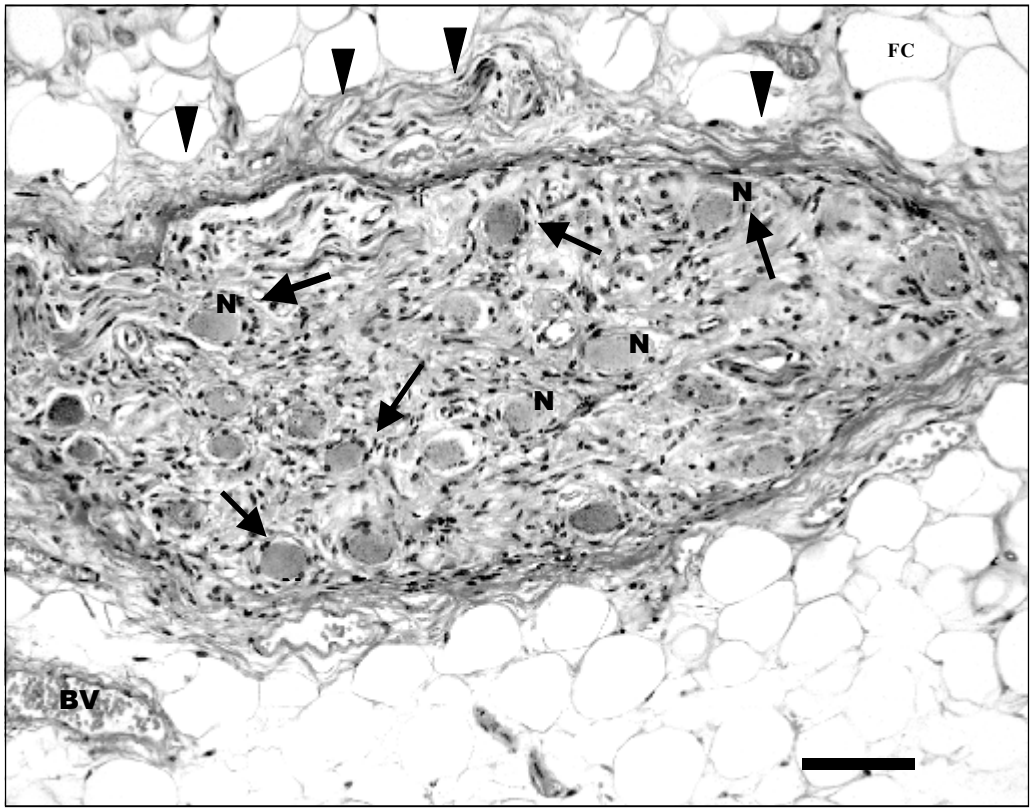

Fig. 1. **Elongate intracardiac ganglion from 78 years of age human heart**

Note that the ganglion is surrounded by thick connective tissue capsule (arrow heads). Neurons (N) are sparse. Nuclei of satellite cells (arrows) are seen around neuronal somas. Ganglion is embedded in fat cells (FC) of the epicardium. Blood vessel (BV) is filled with red blood cells. Scale bar: 0.1 mm.

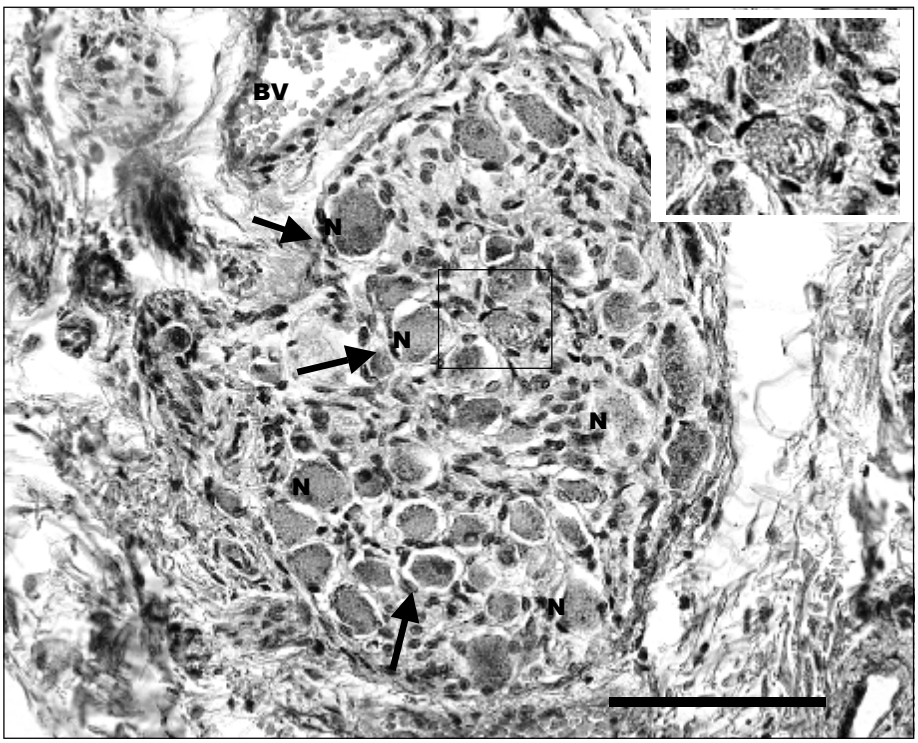

Fig. 2. **Oval shaped intracardiac ganglion from a 20-day-old infant**

Neurons (N) are closely packed. Note that some neurons are surrounded by five and more satellite cells nuclei (arrow) surrounding their somas. A boxed area (enlarged in upper right corner) represents a neuron with two nucleoli. Blood vessel (BV) is filled with red blood cells. Scale bar: 0.1 mm.

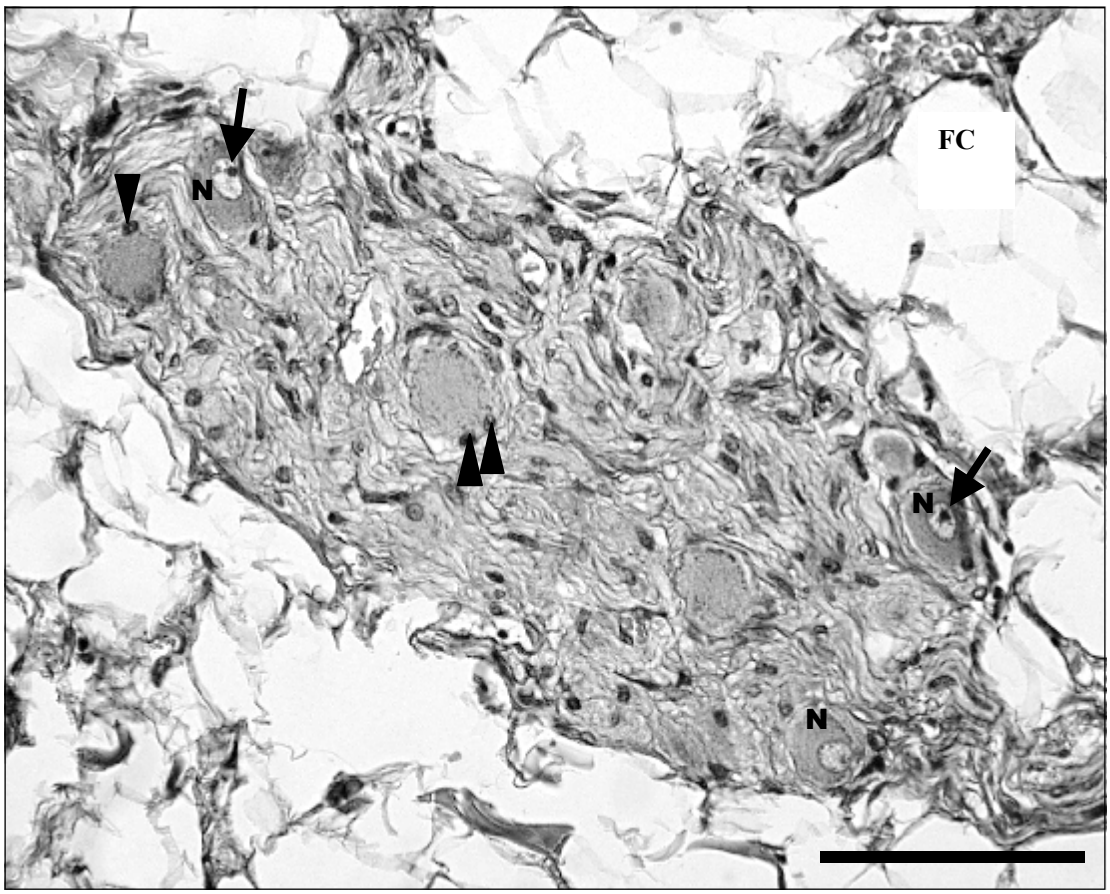

Fig. 3. **Elongated cardiac ganglion from a 61-year-old human heart**

Neurons are oval shaped having eccentrically located nucleus (N) with dense nucleolus (arrow). Note that neuronal cells are sparse. Nuclei of satellite cells (arrow head) are seen around neuronal cell somas. Ganglion is embedded in fat cells (FC) of epicardium. Scale bar: 0.1 mm.

The average frequency of satellite cells was 21±1 μm in infant group, 38±2 μm in adult and 40±2 μm in aged group. Differences between infants and other two groups were statistically significant (Fig. 4D).

The ganglion area was in the infant group 0.031± 0.006 mm^2, in the adult 0.097±0.017 mm^2 and in the aged group 0.083±0.021 mm^2 (Fig. 5A). Statistically significant difference was between infant and adult groups, $p<0.05$. Packing density of neurons was 700±70 mm^2 in the infant group, 230±40/mm^2 in the adult and 230 ± 30/mm^2 neuronal profiles in the aging group (Fig. 5B). The percentage of ganglion area occupied by neuronal bodies was 21.3±0.9%, 13.7± 0.6%, and 16.3±1.1% in the infant, adult and aging groups, respectively (Fig. 5C). Differences between the infant group and two other groups were statistically significant, $p<0.05$.

Discussion

Present study reveals that, in human cardiac ganglia, the somata of neurons increase in size with age. We found that the area of the neurons in adult cardiac ganglia took 48% larger area and in aging 74%, as to compare with infants. Long axis length was 27% longer of adult and 58% longer of aging human intracardiac ganglion neurons, as compared to that of infants. Together with growth of the nerve cell soma the nuclear area increased with age 28 and 76%, correspondingly in adult and aged group. Previous studies on cultured cardiac ganglionic neurons from different postnatal stages of rats showed that neurons both soma and nucleus were significantly larger in later stages (34). For comparison, in the nerve plexus of human esophagus a decreased number of neurons was accompanied by an increased neuronal size (28). The area of nerve cell nucleus from human colon was also found greater in old individuals as compared with infants (23).

We have found few neurons in infant ganglia containing two nucleoli. While up to 36% of the ganglionic neurons contained couple of nucleoli in the dog hearts

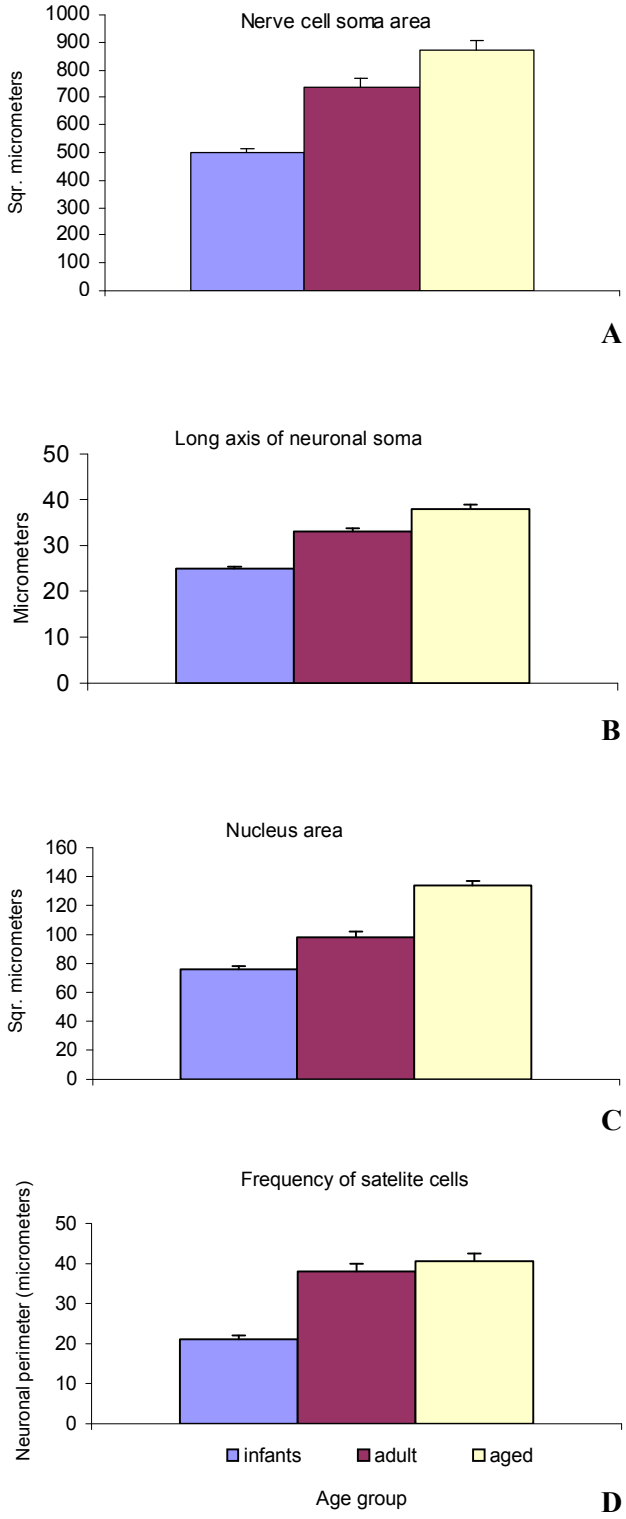

Fig. 4. **Histograms showing the distributions of neuronal soma area (A); neuron soma long axis length (B); nucleus area (C) and satellite cell frequency (D) in three different age groups**

Data are expressed as mean ± SEM. Differences between infant and both other groups: adult and aged were statistically significant when $p<0.05$.

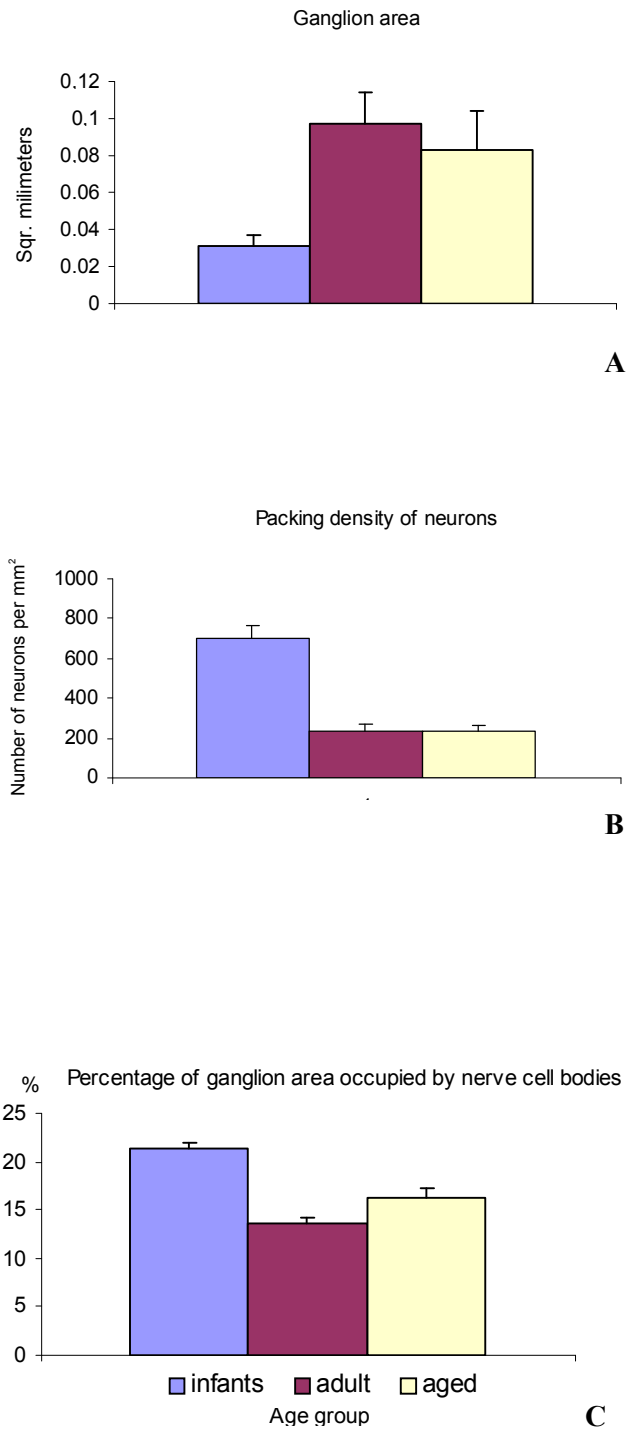

Fig. 5. **Histograms showing the ganglion area (A), statistically significant difference was between infant and adult group when $p<0.05$; neuronal packing density (B) and percentage of ganglion area occupied by nerve cell bodies (C) in different age groups. In B and C differences between infant and both other groups: adult and aged were statistically significant when $p<0.05$.**

Data are expressed as mean ± SEM.

(12). We found no neurons containing multiple nucleoli in the adult or ageing human hearts.

Shape factor equals 1 in a perfect circle, less than 1 means oblong shape (35). About 85% of neurons from superior cervical ganglion of sheep had the form factor greater than 0.9 (35). The form factor of neuronal soma in our study does not vary significantly between all three age groups, meaning that age does not have influence on it.

The estimated frequency of satellite cells around neuronal cell body was smaller in adult and aging individuals, as compared to infants. That could be because of growth on nerve cell soma in size.

The average area of the infant hearts ganglia was tree times smaller than of adult and aged human. Earlier it was estimated that the majority of intrinsic cardiac ganglia both in humans and animals occupied an area ranging from 0.01 to 0.17 mm^2 (36). The present study showed that the cardiac ganglia had better-defined connective tissue capsule in adult and aged human hearts as compared to infant. Infant ganglion capsule was poorly noticeable or absent. Growth of connective tissue was reported in the intrinsic rat cardiac ganglia, capsule grew thicker in old subjects as well as the septa, because both type I collagen and elastic fibers were more numerous (23, 29). An increase in size of neuronal soma is often contemporaneous with decreased number of neurons inside the ganglia (20–22, 24, 29). During our study we found 68% decrease of neuronal packing density with age in adult and old individuals as compared with infants. Also the percentage area occupied by nerve cell bodies inside the ganglia decreased by 39% in adult hearts and by 24% in aged. For comparison, it is noteworthy that the significant reduction (60%) of neurons was reported in human heart (30); in human colon loss of neurons was 37% (23), in human small intestines 34% (20–22, 24, 29) and 40-60% in guinea pig small intestine (21). In human esophagus neurons decreased in number from 22% to 62% along the esophagus after 70 years of age (28). Dramatic loss of neurons seems to be compensated by an increased size probably aiming to maintain a function innervation. However, decreased density may affect heart function, due to a decrease in axonal projections. The mechanisms behind age-related neuronal atrophy are unclear, but may be due to reduced synthesis or availability of target-derived neurotrophic factors (37).

Routine pathologic examination of the heart does not include assessment of the cardiac ganglia (38). It was reported, that cardiac neurons display striking pathological changes during cardiac diseases: loss of Nissl substance in ganglion neurons in patients with diabetes atrophy (38) and in ischemic hearts 35 percent of cardiac neurons displayed inclusions and were markedly enlarged (66×54 μm vs. 40×34 μm for normal neurons). A severe loss of cardiac neurons has been reported in Chagas's disease (39). The pathological changes in the human intrinsic cardiac nervous system have implications with respect to the functional regulation of cardiac functions in various diseases (3). Assessment of age-related changes in hearts not having cardiologic pathology might give a clue for differentiation between disease caused-changes and senescence.

Conclusions

In conclusion, the present study demonstrates significant increase of ganglia, neuron soma and nucleus size with age, accompanied by decreased frequency of satellite cells, neuronal packing density and the area, occupied by neuronal cell somas in the cardiac ganglia from adult and aged humans, compared to infants.

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Žmogaus širdies nervinių mazgų sandaros pokyčiai, susiję su amžiumi

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Raktažodžiai: žmogaus širdis, mazgai, neuronai, morfometrija, amžius.

Santrauka. *Darbo tikslas.* Ištirti žmogaus širdies epikardinio nervinio rezginio nervinių mazgų struktūros pokyčius, susijusius su amžiumi.

Tyrimo medžiaga ir metodai. Tyrimui panaudoti 40 nervinių mazgų, paimtų iš kūdikių, suaugusiųjų ir pagyvenusių žmonių širdžių, parafininiai pjūviai, nudažyti Pikro-Malori metodu. Naudojant kompiuterinę vaizdų

analizavimo programą „Sigma Scan Pro 5.0“, atlikti šie matavimai: nervinio mazgo plotas, neuronų su branduolėliais plotas, neuronų ilgosios ašies ilgis ir perimetras; neuronų branduolio plotas; apskaičiuotas neuronų formos faktorius. Taip pat apskaičiuotas neuronų tankis mazge bei jų užimamas plotas; satelitinių ląstelių, tiesiogiai apsupusių neurono kūną, skaičius ir jų santykinis tankis.

Rezultatai. Su amžiumi padidėjo nervinių mazgų, neuronų kūnų ir branduolių plotas. Neuronų tankis mazge žymiai sumažėjo, tačiau neuronų užimamas plotas mazge sumažėjo nereikšmingai. Satelitinės ląstelės buvo tankiau išsidėsčiusios apie naujagimių neuronus. Neuronų formos faktorius nekito įvairaus amžiaus grupėse.

Išvada. Žmogaus nervinių mazgų sandara su amžiumi žymiai kinta.

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