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The AChE-positive ganglia in the trachea and bronchi of the cat

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The tracheal and bronchial parasympathetic ganglia in the cat were studied using the histochemical-tiocholine method of Koelle and Friedenwald and histological techniques. Intensively stained AChE-positive nerve structures, i.e., ganglia and nerve fibres on the wall of the trachea and bronchi, were observed. The ganglia were situated mainly on the dorso-lateral surface of these organs, but they were also present on the ventral surface. The largest ganglia were found in the vicinity of the vagus nerve branches and on the surface of the tracheal smooth muscle. Numerous ganglia (95–210) of different sizes (40 × 230 μm to 260 × 520 μm) and shapes (spindle, longitudinal, oval, elliptical and multiform) were interconnected by nerve fibres and formed a dense ganglionated plexus. The ganglia forming this nerve structure were located mainly on the level of intercartilaginous spaces. They received the nerve branches from the cervical and the upper thoracic branches of vagus nerve and cervical and upper thoracic segments of the sympathetic trunk. Similar AChE-positive ganglionated plexus containing 28–33 ganglia connected by nerve fibres was observed on the posterior wall of the bronchi.

Histological investigations confirmed the presence of fascicles of nerve fibers and nerve cells aggregations in the external membrane of the trachea and bronchi. The ganglia consisted of 2–25 cells on the cross-section. They were located mainly on the level of intercartilaginous spaces and contained (except ganglionic neurocytes, nerve fibres) satellite cells and small blood vessels. All the ganglia had thin connective capsule.

key words: tracheal and bronchial ganglia, cat, AChE

INTRODUCTION

The autonomic innervation of the respiratory system is of interest to many researchers. Investigations have been concerned with morphology and physiology of the tracheal and bronchial ganglia in different species of animals, e.g., rat [10, 16, 26], guinea pig [3, 6–8, 13, 23, 24, 28], ferret [2, 9], sheep [25] and man [12]. Examinations in man have shown that the intramural plexus in trachea and bronchi are formed at the end of the embryonic and beginning of the fetal period. The plexus in the external membrane of the trachea and bronchi consists of a dense network of fibers and nerve cells, which are mono- or bipolar in a 14-week old fetus. After that, multipolar cells appear in the intramural plexus [12]. The investigations on parasympathetic innervation of bronchi in a developing sheep showed that the vagal preganglionic neurons, which project to airway smooth muscles, are integrated in the control of breathing before birth [25]. It is known that trache-

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al ganglia are the parasympathetic structures; however they also contain sympathetic and sensory fibers. These problems have been explained with some immunochistochemical techniques [13, 15, 17, 20, 22, 23, 27]. The noradrenergic nerve fibers have been found in the mucosa and in the tracheal smooth muscles around blood vessels, but they have never been observed in the proximity of the ganglionic neurocytes [4].

Although numerous studies have been conducted, there is no detailed report on the tracheal ganglia in the cat — except a congress abstract [19]. Therefore, we decided to investigate the topography, morphology and cytoarchitectonics of the tracheal and bronchial ganglia in the cat to complete the data. Also, a comparative analysis was carried out.

MATERIAL AND METHODS

Studies were performed using five two-months old cats. Both male and female cats were used. The animals were deeply anaesthetized with ether and intraperitoneal injection of Nembutal (20–40 mg/kg). The trachea and bronchi from four cats were exposed using a binocular microscope. Then, the material was rinsed *in situ* with a physiological solution and fixed for 30 min in 10% neutral formalin. Other procedures were done in accordance with the histochemical-thiocholine method [18] adapted for whole-mount preparations [14]. From one animal, tissues were taken for the routine histological examination. The tissues were fixed in 4% neutral formaldehyde, embedded in paraffin and cut into 5–8 μm sections using a microtome. Next, the sections were stained using hematoxylin and eosin and Nissl's techniques.

RESULTS

Histochemical study

Intensively stained cholinergic plexus with numerous AChE-positive ganglia was observed along the all length of the trachea. It was localized mainly on the dorsal surface of this organ (Fig. 1, 2). Individual differences in number (95–210), size (up 40 × 230 μm to 260 × 520 μm) and shape of the ganglia were found. The largest ganglia were located along the right and left side of the trachea. There were about 20 ganglia connected by bundles of nerve fibres. Some of these agglomerations of cells had delicate connections with ramification of vagus nerves (Fig. 3). Moreover, we observed connections of the investigated ganglia with sympathetic structures such as the cervical superior, middle and inferior ganglia. The nerve fibers leaving these ganglia formed on the dorsal surface of trachea plexo-ganglionic structures consisting of many ganglia of different sizes and shapes. They were all connected to each other with nerve bundles (Fig. 2, 4). The tracheal ganglia often had a fusiform shape (Fig. 4, 5), but triangular and star-

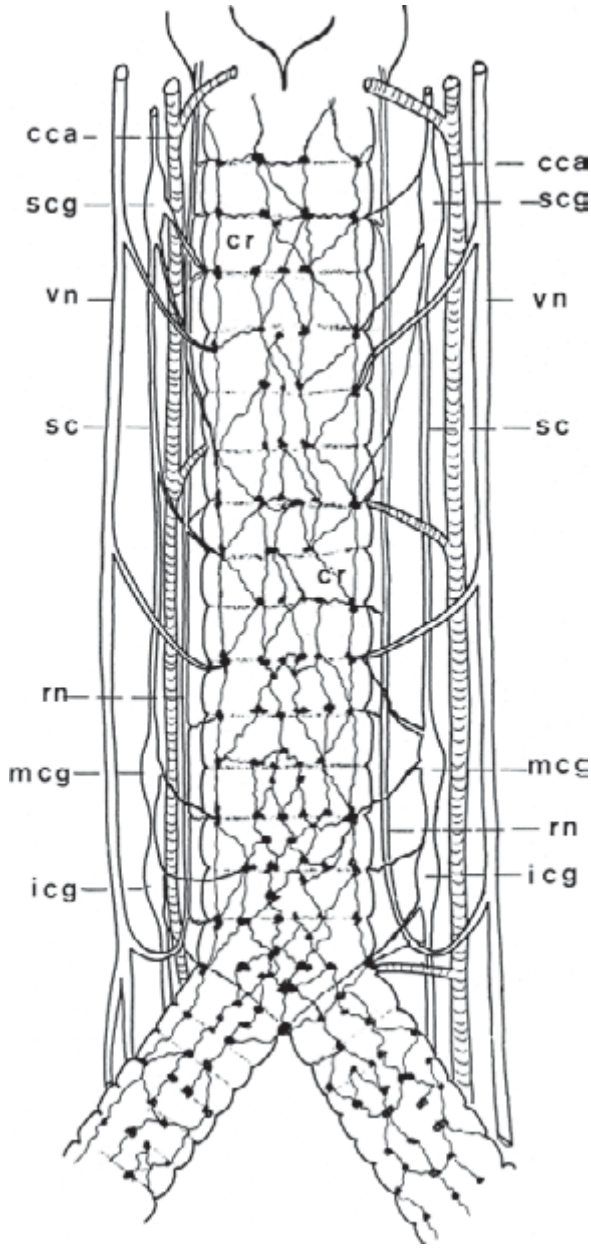

Figure 1. Scheme illustrating the distribution of autonomic ganglia and postganglionic nerve fibres on the dorsal surface of the trachea and bronchi; cca — common carotid artery, cr — cartilaginous rings, icg — inferior cervical ganglion, mcg — middle cervical ganglion, sc — sympathetic chain, scg — superior cervical ganglion, rn — recurrent nerve, vn — vagus nerve.

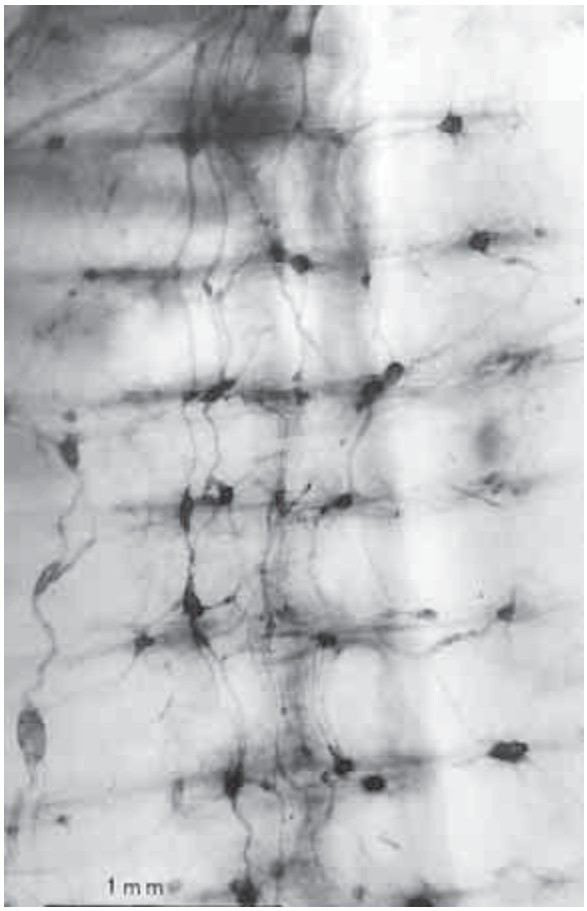

Figure 2. Part of the AChE-positive ganglia and nerve fibres from the posterior wall of trachea — level of 6.th–12th hyaline cartilage.

Figure 4. Different shape of ganglia from the thoracic part of trachea (dorsal wall). Thiocholine method.

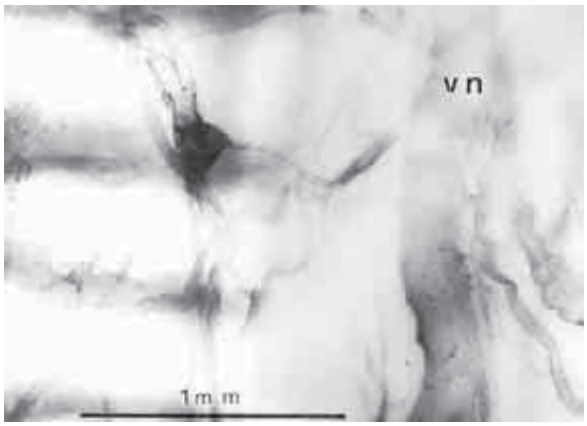

Figure 3. The ganglion from lateral wall of trachea connected with the vagus nerve (vn). Thiocholine method.

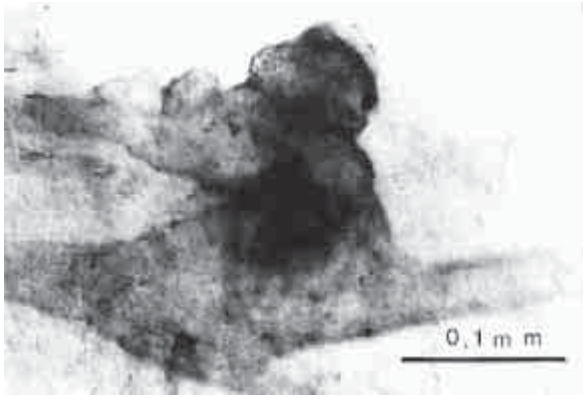

Figure 5. Fusiform, cholinergic ganglion located in the superior part of trachea. Thiocholine method.

shaped structures were also present (Fig. 3). The above-mentioned AChE-positive agglomerations of nerve cells were located mainly on the level of intercartilaginous spaces (Fig. 2).

The cervical part of the trachea had less autonomic ganglia in comparison with the thoracic part. Only 2–5 agglomerations of nerve cells (on the level of the 10th cartilage) in each intercartilaginous space were

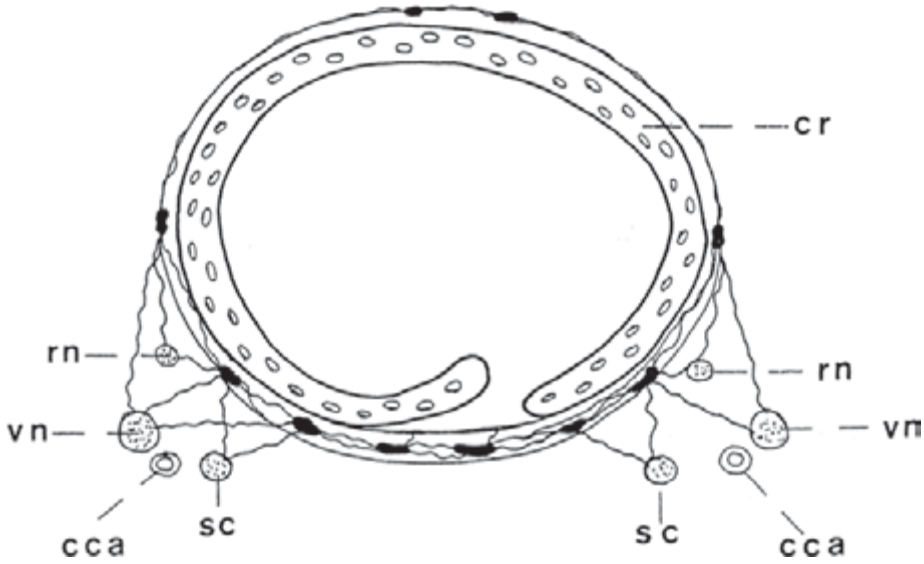

Figure 6. Topographic scheme of parasympathetic structures on the wall of the trachea; cca — common carotid artery, cr — cartilaginous ring, rn — recurrens nerve, sc — sympathetic chain, vn — vagus nerve.

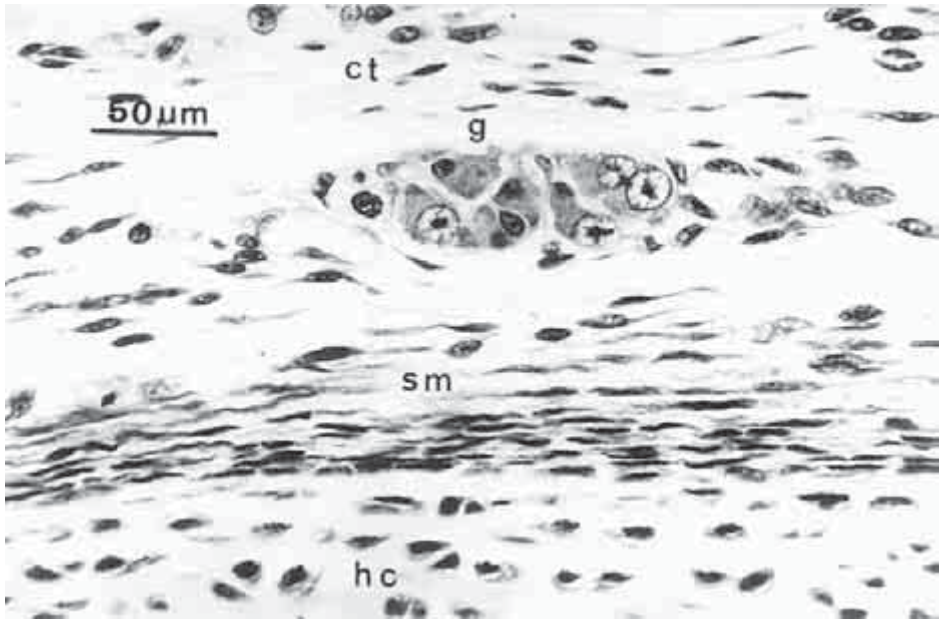

Figure 7. Spindle-shape ganglion from the anterior wall of trachea. Harris hematoxylin; ct — connective tissue, g — ganglion, hc — hyaline cartilage, sm — smoth muscle.

observed. Further, the number of cholinergic ganglia increased from 5–6 (on the level of the 11th cartilage) to 10 (on the level of the 15th cartilage) (Fig. 2). In this way, the density of the AChE-positive structures in the thoracic part of the trachea considerably increased.

The delicate nerve plexus with small AChE-positive ganglia on the ventral surface of the trachea was also observed (Fig. 6, 7). There were 14–32 aggregations of cells situated in the middle of the intercartilaginous spaces. However, the number of the nerve fibers and nerve ganglia on the dorsal surface of the trachea was bigger than that on the ventral surface.

We also observed the AChE-positive nerve fibres and numerous ganglia along the all length of the

bronchi. They formed a characteristic nerve network with aggregations of nerve cells in locations where the fascicles of the nerve fibres crossed each other (Fig. 8). The ganglia were located mainly on the dorsal wall, but they were also present on the ventral surface. We observed 23 to 38 aggregations of cells of different sizes and shapes. They were all connected with bundles of nerve fibers.

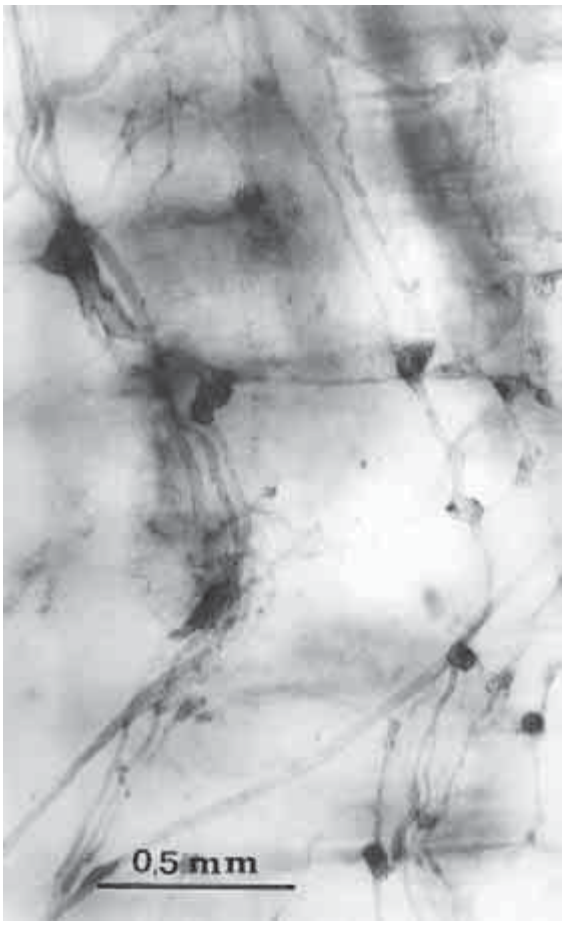

Figure 8. Parasympathetic ganglia from the dorsal wall of bronchi. Thiocholine method.

Histological study

The histological investigations confirmed the presence of numerous ganglia on the dorsal and ventral surfaces of the external membrane of the trachea and bronchi. The ganglia were different in sizes and shapes (spindle, longitudinal, oval, elliptical and multiform). We found that the smaller and bigger ganglia, respectively, consisted of 2–9 and 10–25 nerve cells on cross-sections of the dorsal surface of the trachea. They were localized between the connective tissue and the smooth muscle of the trachea (Fig. 9, 10). Sometimes flat and longitudinal ganglia with only one layer of nerve cells were observed (Fig. 9). The ganglia contained (except neurocytes) nerve fibers and blood vessels. The neurocytes in the tracheal ganglia were distributed regularly on the ganglion and occupied about 70–80% the sectional area. Each neurocyte had one light-stained nucleus with intensively stained nucleoli. The satellite cells with dark stained nuclei were seen among the ganglionic

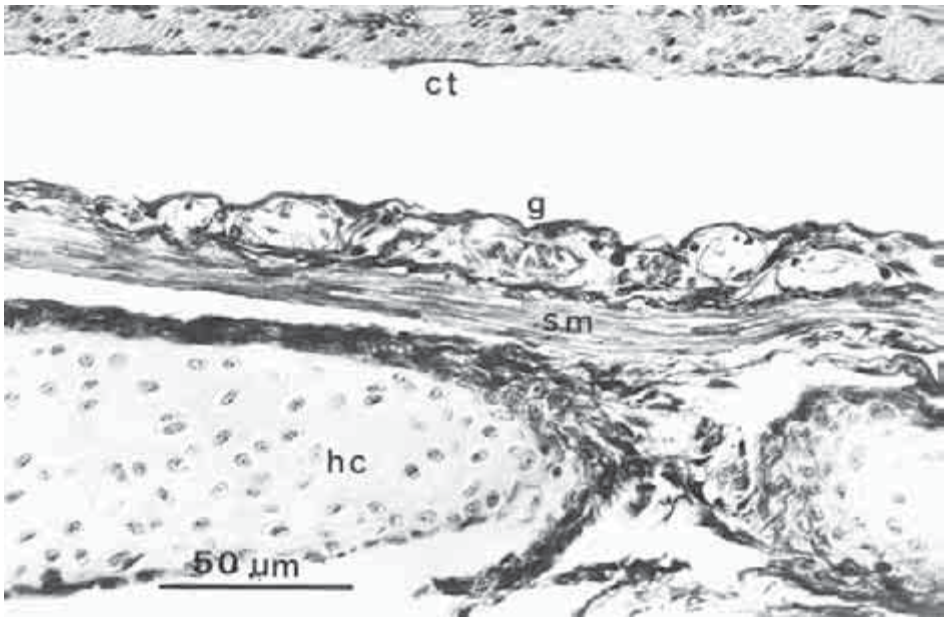

Figure 9. Elongated ganglion from the posterior wall of the trachea. Harris hematoxylin; ct — connective tissue, g — ganglion, hc — hyaline cartilage, sm — smoth muscle.

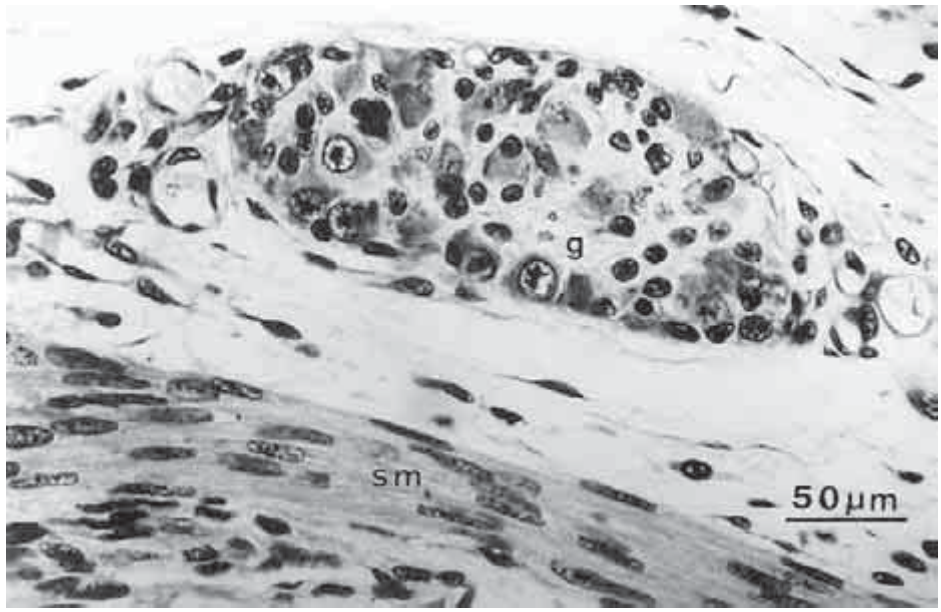

Figure 10. Large oval ganglin with 25 ganglion cells on the cross-section of the dorsal wall of the trachea. Harris hematoxylin.

neurocytes (Fig. 7, 10). The size of the ganglionic cells fluctuated between 16 μm and 31 μm. The all ganglia had thin connective capsules. The ganglia from the anterior surface of the trachea were smaller than the ganglia from the posterior surface. They usually had oval or spindle shapes and consisted of only 2–5 cells on the cross-section (Fig. 7). Also, these ganglia had thin connective capsules.

The bronchi ganglia located on the external membrane had only 2–5 cells on the cross-section and a connective capsule. Their morphology was similar to that of the ganglionic cells from the trachea. The sizes of these neurocytes ranged from 15 μm to 30 μm.

DISCUSSION

The parasympathetic fibers in the trachea originate from the vagus nerve. Most of them enter through the recurrent laryngeal nerves [5]. The parasympathetic innervation of the investigated species is similar. However, we observed a direct connection between the trunk of the vagus nerve and the tracheal plexus. Previous investigations showed that sympathetic nerve fibers of the trachea originate from the cervical superior ganglion and the stellar ganglion [20, 27]. Experimental examinations showed that sympathetic fibers supply the trachea with the recurrent laryngeal nerve via the anastomoses between the sympathetic chain and the vagus nerve [5]. In our dissection observations, we noticed a direct connection between some tracheal ganglia and the branches from the cervical ganglia (superior, medial and inferior).

The sensitive supply neurons in the trachea and bronchi were found in the nodose and jugular vagal ganglia and in the upper thoracic dorsal root ganglia [15, 20, 27].

The morphology of neurocytes and tracheal ganglia was most often examined in the guinea pig [3–5, 21] and rat [10]. Most of the neurocytes were multipolar with a number of short processes. The number of tracheal ganglia in the examined species was different. In the guinea pig there were 166––327 ganglia (average 222), and in the rat 63–78 (average 70). In our examinations, there were 95 to 210 ganglia in the cat. These results suggest that there is a correlation between the number of ganglia and the animal size, and between the number of nerve cells and the size of the respiratory system. This can be confirmed with a larger amount of data.

Two plexuses on the dorsal wall of the trachea were found in the rat. The first (more delicate structure) was in the cervical part, and the second (80% of all ganglia) was in the thoracic part [10]. We observed that the AChE-positive ganglia and the nerve fibers in the thoracic part were denser than that in

the cervical part. But, the difference was not as big as that in the rat. On the other hand, in the cat, except the "plexo-ganglionic structure" on the dorsal surface of the trachea, we found the second, but smaller and more delicate "plexo-ganglion" on the ventral surface of this organ. The similar situation was observed in the ferret [2].

The localization of the tracheal and bronchial ganglia mainly on the posterior surface of these organs is connected with the smooth muscle activity [1] and the presence of mucous glands in this part of the mucous membrane [11].

Many investigations were concerned with physiological differentiation of the neurocytes of the tracheal and bronchial plexuses. These ganglia were parasympathetic structures and did not show catecholamine fluorescence. But in guinea pig some tracheal neurons were dopamine beta-hydroxylase immunoreactive [4]. However, the sympathetic fibers from the cervical ganglia supply the blood vessels, smooth muscles and the mucosa but were not found around or in the proximity of neurons of the tracheal ganglia [4]. Some investigations were concerned with the presence of VIP in postganglionic parasympathetic fibers, where they modulate cholinergic neurotransmission by predominantly influencing the postganglionic nerves. It has also a small additional effect on ganglionic transmission [17, 22]. At the ends of the preganglionic nerve fibers, the presence of SP was detected [15, 20, 24]. Examinations on the tracheal innervation of the guinea pig showed the presence of non-adrenergic and non-cholinergic neurons. They are localized in the oesophageal myenteric plexus and their axons supply the trachea [13, 23]. Moreover, two types of neurons were found in the tracheal ganglia using electrophysiological methods. It is only in the ganglia that neurons lacking synaptic inputs (90%) (associated with recurrent nerve trunks) and neurons with fast excitatory synaptic potentials are located very close to the smooth muscle [21]. Investigations with the retrograde tracing showed that in airway ganglia in the ferret the morphology of spiking and nonspiking neurons is remarkably similar despite the electrophysiological differences [9].

The electron microscopic investigation of the guinea pig tracheal ganglia showed the similarity of ganglionic cells with other parasympathetic and sympathetic nerve cells [3]. All the surfaces of neurons were covered with a complete satellite layer. There were delicate blood vessels, myelinated and nonmyelynated nerve fibres in side the ganglia.

In our histological investigation, we also observed nerve fibres and blood vessels between the ganglionic cells. Considering the histological organization, it seems there is a general similarity between the tracheal and bronchial ganglia and other parasympathetic ganglia.

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