Z. Anat. Entwickl.-Gesch. 143, 215—222 (1974)

The Fine Structure of the Pulmonary Stretch Receptor in the Rat*

M. v. Düring, K. H. Andres and J. Iravani

Anatomisches Institut der Ruhr-Universität Bochum
and Institut für Lungenfunktionsforschung Bochum

Received February 19, 1974

Summary. In rats the 3rd to 6th bronchi, measuring 500–700 μ in diameter during inspiration, were investigated by light and electron microscopy. The histological appearance of these bronchi is comparable to that of medium sized bronchioles of larger animals.

The branched and lanceolate terminals are associated with the connective tissue of the lamina propria and the smooth muscle cell layer. In this way the terminals are bound to the myoelastic system of the bronchial wall. The myelinated afferent fiber is branched and the diameter measures about 4–6 microns. Besides afferent nerve terminals these are numerous efferent endings on the smooth muscle basement laminae.

It is supposed that the described receptor represents the pulmonary stretch receptor of the Hering Breuer reflex.

Key words: Lung—Stretch receptors—Light microscopy—Electron microscopy—Rat.

Zusammenfassung. Lappenbronchien (3./6.) (Durchmesser bei mittlerer Inspirationslage 500–700 μ) von männlichen und weiblichen Wistarratten wurden licht- und elektronenmikroskopisch untersucht. Diese Bronchien entsprechen im Wandbau den mittleren Bronchioli größerer Säugetiere.

Freie, verzweigte lanzettförmige Terminalfasern werden im Bindegewebe der lamina propria beschrieben. Sie sind in das elastisch muskuläre System der Bronchuswand eingebaut. Die zugehörige markhaltige afferente Nervenfaser ist verzweigt und hat einen Durchmesser von 4–6 μ. Außerdem werden efferente motorische Endigungen an der glatten Bronchialmuskulatur beschrieben.

Die freien lanzettförmigen und verzweigten Terminalfasern sind möglicherweise Dehnungsrezeptoren für den Hering Breuer Reflex.

Introduction

Several morphological studies have been published concerning the innervation of the lung (Larsell, 1921; Larsell and Dow, 1933; Elftmann, 1943; Spencer *et al.*, 1964; Bensch *et al.*, 1965; Blümcke, 1968; Lauweryns *et al.*, 1969, 1972; Fillenz, 1970; Hung *et al.*, 1972). Lauweryns (1972) described intraepithelial receptors in the mucosa of the intrapulmonary airways as "neuroepithelial bodies". The intraepithelial axons are associated with specialized epithelial cells containing many dense granules. This receptor type, discussed as lung irritant receptor (Hung *et al.*, 1973), is usually located at the branching sites of the bronchioles.

Larsell (1923) described another type of afferent nerve terminal located in the smooth muscle bands of the bronchioles which he called "smooth-muscle nerve

* This study has been supported by the "Verein zur Untersuchung von Einwirkungen der Luftverschmutzung auf die Volksgesundheit e.V. Bochum" and the Deutsche Forschungsgemeinschaft (SFB Bionach).

spindle". The afferent nerve fiber is myelinated. On the basis of physiological data, Widdicombe (1953) suggested that the slowly adapting pulmonary stretch receptor of the Hering Breuer reflex is in close contact with the smooth muscle cells of the pulmonary airway, while Weidmann *et al.* (1949) suggested this receptor to be situated below the pleura.

The present report concerns the morphology of a mechanoreceptor in the lamina propria of the bronchial wall in the rat.

Materials and Method

Adult male and femal Wistar rats were used. The animals were anaesthetized by phenobarbital injected intraperitoneally. The trachea was then cannulated, a tracheal cannula was inserted and the two lungs were inflated to a normal size. The tube was then closed so that the volume of the lungs remained constant. The animal was killed, the chest opened and the trachea and the lung removed. The heart lobe was prepared as previously described by Iravani (1971). After fixation for 2 hours in 3.5% buffered glutaraldehyde the tissue was dissected for microscopic investigation. The results presented here were obtained from the middle section of the bronchus 3./6. (see Iravani, 1971). The wall structure of this bronchus is identical to a bronchiolus of medium size of larger animals. The pieces were rinsed in phosphate buffer, postfixed in 4% osmiumtetroxide, dehydrated in graded ethanol and embedded in Araldite.

Serial sections were made with a Porter Blum MT I. The ultrathin sections were cut on an Ultrotome (LKB III) with a diamond knife, double-stained with uranyl acetate and lead citrate Reynolds 1963 and examined with an electron microscope (Philips 300).[1]

Light Microscopy

A cross section through a small bronchus (3./6.) shows the single layered epithelium with ciliated cells, goblet cells and some brush cells. The lamina propria consists of reticular and collagen connective tissue with many elastic fibers. They are most prominent just below the basement lamina as seen in Fig. 1. Next to the mucosa is a layer of smooth muscle cells. Numerous elastic fibers are intimately associated with the smooth muscle cells. A network of blood vessels and lymphatic vessels accompanies and penetrates the myoelastic layer. Below or between the smooth muscle layer a number of nerve fiber bundles are to be seen. These consist mainly of unmyelinated axons but among them between 1 to 5 myelinated fibers with a diameter of about 4–6 microns are often observed. Serial sections of these bundles show that the perineural sheath is more prominent in those small nerves which lie below the smooth muscles. Between the muscle cells, the lamellation of the perineural sheath becomes more and more indistinct. Before it looses its myelin sheath the axon enlarges and is found to contain a great number of mitochondria in the axoplasm.

In this region the regular arrangement of the smooth muscle cells changes in a typical manner. Much more reticular connective tissue and elastic fibers penetrate the layer of the smooth muscle cells.

Observing the lamina propria and the muscle fibers it is possible to distinguish ovoid light profiles of about 2–5 microns in diameter. Electron micrographs show that these profiles are afferent nerve terminals.

1 We thank Mrs. Brigitte Decker for skilfull technical assistance.

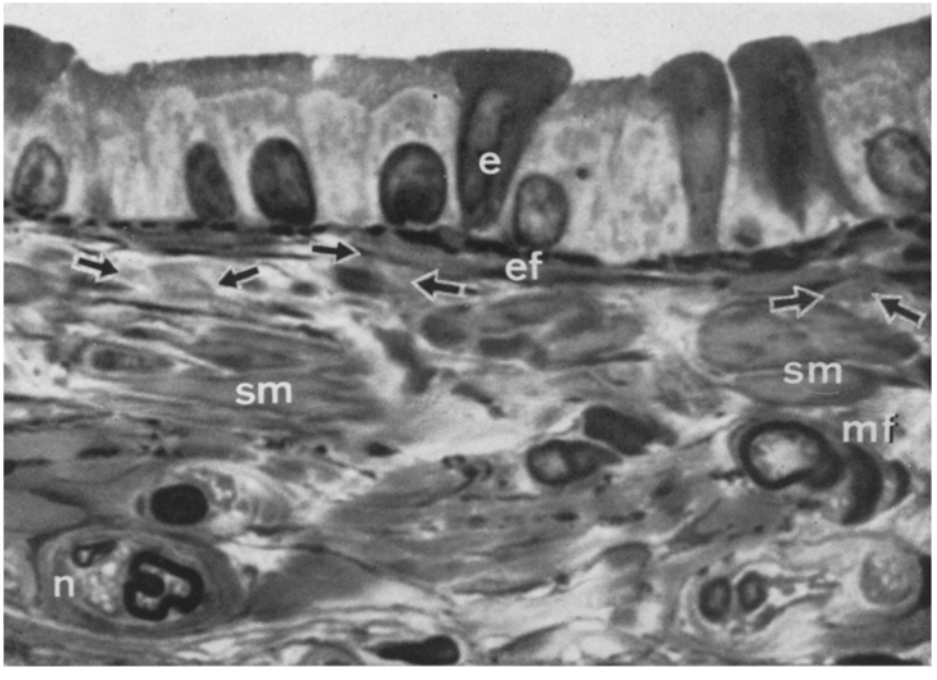

Fig. 1. Cross section through the wall of a rat bronchus (3./6.) with the afferent myelinated nerve fiber (*mf*) of the pulmonary stretch receptor. Terminals (arrows) of the branched receptor axon lie between the connective tissue of the lamina propria and the smooth muscle cells (*sm*). The terminals of the receptor axon are difficult to recognize. Respiratory epithelium (*e*), elastic network (*ef*), a small nerve (*n*) is located in the collagenous layer of the bronchus wall. Semithin section: × 1700

Electron Microscopy

After losing the myelin sheath, the axon splits up into several branches and terminates as branched free lanceolate terminals (Andres, 1966). These terminals are located in the connective tissue between the lamina propria and the layer of smooth muscle cells and are oriented parallel to the long axis of the bronchus. The receptor axon looses its Schwann cell covering and is protected only by its basement laminae. Finally, as observed in cross sections, the two poles of the spindle-shaped axon terminal are elongated. They are not completely covered by the basement laminae (Fig. 2), but are in direct contact with the surrounding elastic and collagen fibers of the connective tissue. In some cases the elastic fibers in contact with the receptor terminal can be followed to the basement laminae of the smooth muscle cell. The two elongated poles or the fingerlike processes of the enlarged axon terminal contain filamentous material similar to the receptor matrix. The receptor matrix is interspersed with clear and granulated vesicles and associated with lamellated corpuscles and lysosomal elements. A great number of round and ovoid mitochondria are embedded in the center of the terminal. In the proximal region the receptor axon contains many axoplasmatic structures such as a network of microtubules, axoplasmatic reticulum and

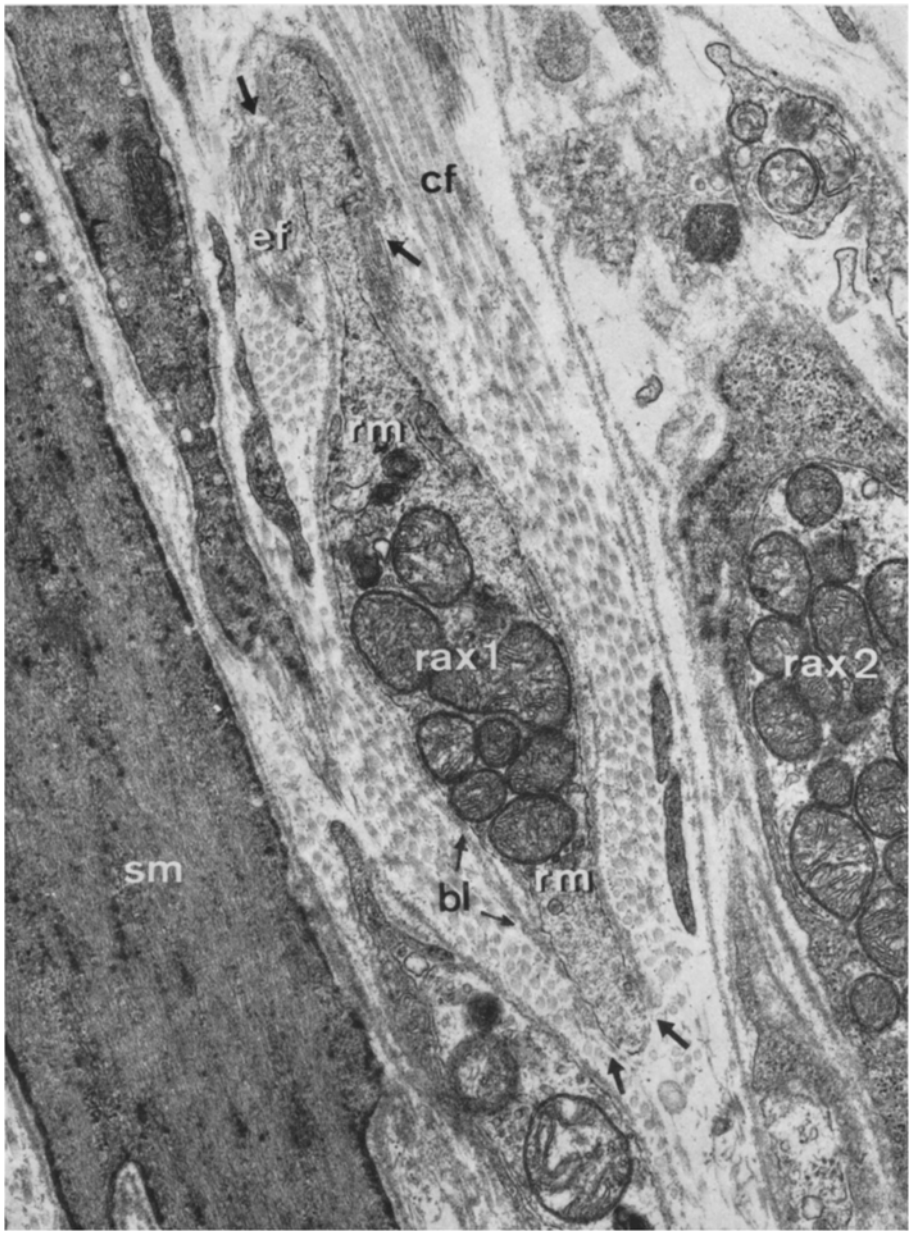

Fig. 2. A cross-sectioned lanceolate terminal (*rax 1*) of the pulmonary stretch receptor. The edges of the "lancet" show tight contact to collagen and elastic fibers (arrows). The basement laminae (*bl*) does not envelope the axon terminal completely. *Rax 2* represents another branch of the receptor axon. Receptor matrix (*rm*), elastic fibers (*ef*), collagen fibers (*cf*), smooth muscle cell (*sm*). × 25000

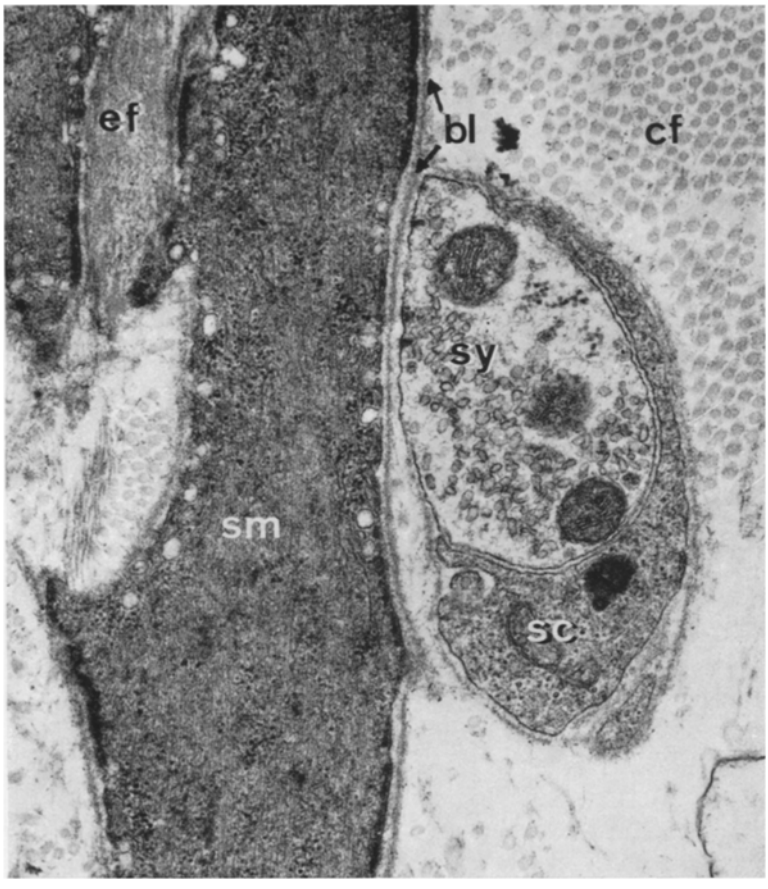

Fig. 3. Synaptical contact (*sy*) of an efferent nerve fiber with a smooth muscle cell (*sm*) within the receptor field of the pulmonary stretch receptor. Schwann cell (*sc*), basement laminae (*bl*), elastic fiber (*ef*), collagen fibers (*cf*). × 25000

some neurofilaments. These structures can be followed well into the lanceolate terminal; however they gradually decrease and disappear in the receptor matrix.

Motor Innervation

In contrast to the silver technique, electron microscopy allows the ready identification of afferent nerve terminals from efferent nerve terminals. All the efferent terminals belong to unmyelinated axons and innervate the smooth muscle cells. The axons running between the smooth muscle cells often enlarge at intervals and within these enlargements an abundance of clear vesicles is usually present. An additional important feature may serve to differentiate the efferent nerve terminal. At the point, where the efferent fiber has its "synapse", the Schwann cell cytoplasm retracts and the basement laminae of the nerve terminal fuses with the basement laminae of the smooth muscle cell (Fig. 3).

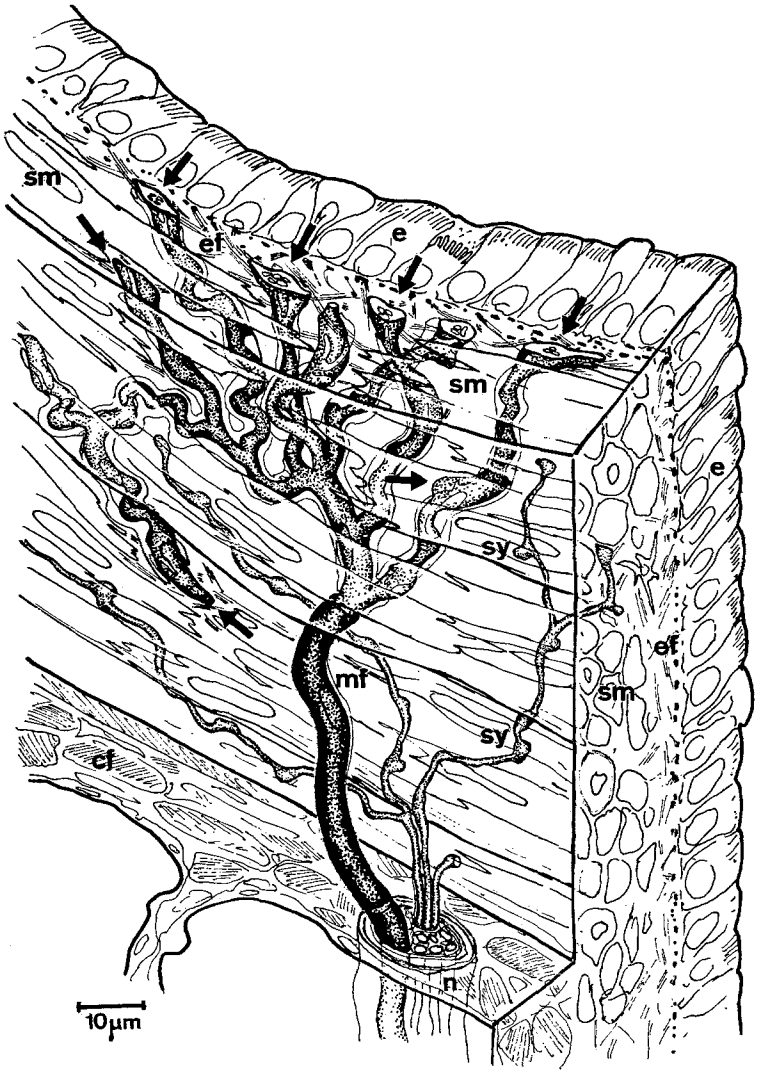

Fig. 4. Schematic representation of a segment of a rat bronchus wall with the nerve endings (arrows) of the pulmonary stretch receptor. The lanceolate terminals are anchored within the reticular connective tissue below the respiratory epithelium (*e*). The smooth muscle cell layer (*sm*) is interrupted in the receptor field of the pulmonary stretch receptor. Efferent nerve terminals (*sy*) derive from nonmyelinated nerve fibers. Afferent myelinated nerve fiber (*mf*) of the receptor, elastic network (*ef*), a small nerve (*n*), collagen fiber (*cf*)

Discussion

The pulmonary stretch receptor described in this paper is a free branched and lanceolate receptor. Based on our comparative studies concerning the fine structure of cutaneous mechanoreceptors, we can classify this pulmonary receptor as belonging to the main group of stretch receptors such as the Ruffini corpuscle

(Chambers *et al.*, 1972) and the Golgi tendon organ (Houk *et al.*, 1967; Stuart *et al.*, 1970; Schoultz *et al.*, 1972). Common morphological criteria of these receptors are the very close contact of the nerve terminals to collagen and elastic fibers and the orientation of their axis in the direction of the most frequent stretches in the corresponding organ (Andres, v. Düring, 1973).

With regard to the localization of the free branched and lanceolate terminals and to their integration into the myoelastic system of the bronchus wall (Fig. 4) one can assume that the described receptor represents the pulmonary stretch receptor of the Hering Breuer reflex. This reflex was described by Hering and Breuer (1868). The stretching of the bronchial wall during inspiration has an influence on the receptor endings due to increased tension in the myoelastic system. The tension of the bronchial wall during the inspiration is an important event for the response pattern of the receptor.

On the other hand, the sensitivity of the nerve terminals may be modified by any change of the smooth muscle tonus of the bronchus.

The axon diameters of the branched and lanceolate receptor endings are of the same magnitude as those of the afferent axons reported by Paintal (1953b) and Widdicombe (1953) during physiological investigation of the pulmonary stretch receptors (Hering Breuer reflex).

Based on the comparative morphology of cutaneous mechanoreceptors, Andres (1973) suggested that similar structural nerve endings responsive to identical mechanical stimulation might also be present in the viscera. The present paper confirms this point of view with regard to the branched lanceolate terminal.

References

Andres, K. H.: Über die Feinstruktur der Rezeptoren an Sinushaaren. Z. Zellforsch. **75,** 339–365 (1966)

Andres, K. H.: Morphological criteria for the differentiation of mechanoreceptors in vertebrates. Symposium Mechanorezeption, Bochum 1973 (in press)

Andres, K. H., Düring, M. v.: Morphology of cutaneous receptors. In: Handbook of Sensory Physiology (A. Jqgo, ed.), vol. II, p. 3–28. Berlin-Heidelberg-New York: Springer 1973

Bensch, K. G., Gordon, G. B., Miller, L. R.: Studies on the bronchial counterpart of the Kultschitzky (Argentaffin) cell and innervation of bronchial glands. J. Ultrastruct. Res. **12,** 668–686 (1965)

Blümcke, S.: Experimentell-morphologische Untersuchungen über die efferente Bronchusinnervation I. Plexus peribronchialis. Beitr. path. Anat. **137,** 239–286 (1968)

Chambers, M. R., Andres, K. H., Düring, M. v., Iggo, A.: Structure and function of the slowly adapting type II mechanoreceptor in hairy skin. Quart. J. exp. Physiol. **57,** 417–445 (1972)

Elftmann, G.: The afferent and parasympathic innervation of the lungs and trachea of the dog. Amer. J. Anat. **72,** 1–27 (1943)

Fillenz, M.: Innervation of pulmonary and bronchial blood vessels of the dog. J. Anat. (Lond.) **106,** 449–461 (1970)

Hering, E., Breuer, J.: Die Selbststeuerung der Atmung durch den Nervus vagus. S.-B. Adad. Wiss. Wien **58,** 909–937 (1868)

Houk, J., Hennemann, E.: Responses of tendon organ to forces applied to muscle tendon. J. Neurophysiol. **30,** 466–481 (1967)

Hung, K. S., Hertwech, M. S., Hardy, J. D., Coosli, J. D.: Innervation of pulmonary alveoli of the mouse lung. An electron microscopic study. Amer. J. Anat. **135,** 477–496 (1972)

Hung, K. S., Hertwech, M. S., Hardy, J. D., Coosli, J. D.: Ultrastructure of nerves and associated cells in bronchiolar epithelium of the mouse lung. J. Ultrastruct. Res. **43,** 426–437 (1973)

Iravani, J., Schlüter, K. G.: In vitro Untersuchungen der Bronchomotorik der Ratte. Pneumologie **144,** 253–265 (1971)
Larsell, G.: Nerve terminations in the lung of the rabbit. J. comp. Neurol. **33,** 105–131 (1921)
Larsell, G.: The ganglia, plexuses and nerve terminations of the mammalian lung and pleura pulmonalis. J. comp. Neurol. **35,** 97–132 (1923)
Larsell, G., Dow, R. S.: Innervation of the human lung. Amer. J. Anat. **52,** 125–146 (1933)
Lauweryns, J., Peuskens, J.: Argyrophil (Kinin and amine producing?) cells in human infant airway epithelium. Life Sa. **8,** 577–586 (1969)
Lauweryns, J., Peuskens, J.: Neuroepithelial bodies (Neuroreceptor or secretory organs?) in the human infant bronchial and bronchiolar epithelium. Anat. Re. **172,** 471–482 (1972)
Lauweryns, J., Peuskens, J., Cokelaere, M., Theunyuck, P.: Neuroepithelial bodies in the respiratory mucosa of various mammals. Z. Zellforsch. **135,** 569–592 (1972)
Paintal, A. S.: The conduction velocities of respiratory and cardiovascular afferent fibres in the vagus nerve. J. Physiol. (Lond.) **121,** 341–359 (1953b)
Reynolds, E. S.: The use of lead citrate at high pH as an electron-opac stain in electron microscopy. J. Cell Biol. **17,** 208–212 (1963)
Schoultz, T. W., Swett, J. E.: The fine structure of the Golgi tendon organ. J. Neurocytol. **1,** 1–26 (1972)
Spencer, H.: The innervation of the human lung. J. Anat. (Lond.) **98,** 599–609 (1964)
Stuart, D. G., Goslow, G. E., Mosher, C. G., Reinking, R. M.: Stretch responsivness of Golgi tendon organs. Exp. Brain Res. **10,** 463–476 (1970)
Weidmann, H., Berde, B., Bucher, K.: Die Lage der vagalen Dehnungsrezeptoren in der Lunge. Helv. physiol. pharmocol. Acta **7,** 476–481 (1949)
Widdicombe, J. G.: The localization of pulmonary stretch receptors in the cat. J. Physiol. (Lond.) **122,** 26–27 (1953)

Dr. Monika von Düring
Anatomisches Institut II
der Universität
Postfach 2148
D-4630 Bochum
Buscheystrasse MA-6/161
Federal Republic of Germany