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The intrapulmonary neuroepithelial bodies after vagotomy: demonstration of their sensory neuroreceptor-like innervation[1]

J.M. Lauweryns and A. Van Lommel

Katholieke Universiteit te Leuven, School of Medicine, Laboratory of Histopathology, 12, Minderbroedersstraat, B-3000 Leuven (Belgium), February 28, 1983

Summary. In the neonatal rabbit, infranodosal vagotomy destroys most of the intracorpuscular nerve endings of the pulmonary neuroepithelial bodies (NEB), while supranodosal vagotomy leaves these nerve endings intact. We conclude that NEB are mainly innervated by sensory neurons whose cell bodies lie in the nodose ganglion of the vagus nerve. These findings support the hypothesis that although secretory in nature, NEB are neuroreceptor structures.

Apart from single neuroendocrine-like cells[2-6], innervated corpuscles of such cells, termed neuroepithelial bodies (NEB) recently have been identified in the intrapulmonary airway epithelium of man[7], several mammalian species[8-11], and amphibians[12,13]. NEB are composed of high cylindrical non-ciliated epithelial cells that contain serotonin[8,14-16] and peptides[17-19], stored in their intracytoplasmic dense-cored vesicles. Their strategic position at airway bifurcations[7,8], their distinct innervation which includes morphologically afferent (sensory) nerve endings[20,21] and the release by the corpuscular epithelial cells of serotonin upon their exposure to hypoxia[22-24], suggest that they may represent some kind of intrapulmonary neuro(chemo)receptor with local secretory activities and apparently sensitive to the composition of the inhaled air.

A condition to be satisfied on assigning a neuroreceptor function to these NEB is an unequivocal demonstration of the sensory nature of their innervation. In this report, we present neuroanatomical evidence obtained after nerve degeneration experiments and indicating that indeed most of the intracorpuscular nerve endings are sensory.

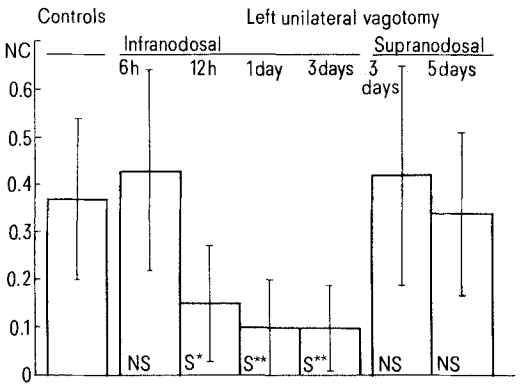
It is classically known that the sensory innervation of most of the thoracic and abdominal viscera, including the lungs[25], originates from pseudounipolar neurons whose cell bodies lie in the nodose ganglion of the vagus nerve[26]. We have thus studied the NEB intracorpuscular nerve endings after section of the vagus nerve either above or below the nodose ganglion, investigating whether the obtained results are compatible with the hypothesis that these nerve endings are sensory (i.e. terminals of processes of neurons whose cell bodies are situated in the nodose ganglion).

Although the anuran lung and its NEB are morphologically very different from the mammalian lung, it may be mentioned that a total vasosympathetic denervation has been carried out on the toad lung[12]. These experiments, however, only demonstrated that the origin of the nerve fibers to the amphibian NEB arises from a source 'extrinsic to the lung'[12].

Material and methods. Left unilateral vagotomy was performed on 17 neonatal rabbits, aged 1-2 weeks. The animals were anesthetized by an i.m. injection of Hypnorm® in doses of 0.05 ml per 100 g b.wt. The left vagus nerve was exposed in the midcervical region.

In 12 rabbits, the nerve was sectioned below the nodose ganglion (infranodosal vagotomy), a short length of it being removed at the same time. The animals were allowed to survive for a time interval varying from 6 h (2 animals) to 12 h (2 animals), 1 day (4 animals) and 3 days (4 animals).

In 5 rabbits, the left vagus nerve was surgically exposed in a cranial direction until the nodose ganglion was reached. The nerve was then sectioned above the nodose ganglion



Comparison of the ratio (NC) of the number of intracorpuscular nerve endings to the number of corpuscular cells in the NEB of control and infranodosally or supranodosally vagotomized left rabbit lungs; various survival times (as indicated on the figure). The SD are in each instance indicated by the brackets. A total number of 134 NEB was studied. Statistical significance: S*, $p < 0.05$; S**, $p < 0.01$; NS, not significant.

(supranodosal vagotomy). The animals were allowed to survive for 3 (3 animals) to 5 days (2 animals). Another 4 rabbits were not operated on, their left lungs serving as controls.

When sacrificed, the animals were reanesthetized. Their lungs were fixed by an intratracheal instillation of ice-cold 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2). The lungs were cut in small cubes, further immersed for another 2 h under vacuum, and postfixed for 1 h in 1% OsO_4 in 0.1 M phosphate buffer (pH 7.2). The biopsies were embedded in Epon and 1 µm sections stained with toluidine blue for a light-microscopic examination. Whenever a NEB was observed, the rest of the block was trimmed and ultrathin (40–60 nm) sections of the corresponding area cut, mounted on copper grids and stained with uranyl acetate and lead citrate for an ultrastructural investigation using a Philips 300 electron microscope at 60 kV.

For each NEB of the experimental and control left lungs, the ratio of the number of visible intracorpuscular nerve endings to the number of transected corpuscular cells (NC ratio) was determined on a single section. The differences in this NC ratio between the control group and the experimental groups were statistically evaluated by means of F-tests. A total of about 130 NEB was studied.

Results (fig.). Compared to the control lungs, the NC ratio of the denervated left lungs remains practically unchanged 6 h after infranodosal vagotomy. 12 h to 3 days postoperatively however, the NC ratio falls significantly in the denervated left lung. Supranodosal vagotomy, on the other hand, does not influence the NC ratio of the left lung by 3–5 days postoperatively.

Of the relatively few nerve endings which remain observable in the NEB after infranodosal vagotomy, several exhibit abnormal morphological characteristics corresponding to degenerating nerve endings. Their morphology will be described in detail elsewhere.

Discussion. The vagus nerve has a heterogeneous composition[26]. It contains parasympathetic preganglionic motor fibers, originating from cell bodies in the brain stem and terminating on peripheral ganglion cells in the walls of the viscera. In addition it comprises sensory fibers formed by the processes of pseudounipolar neurons whose cell bodies are located in the nodose ganglion.

After infranodosal vagotomy, degeneration of nerve endings localized at the level of the respiratory epithelium has been reported by a number of authors[27,28]. As this procedure leaves intact the peripheral postganglionic motor neurons of the vagus, these degenerating nerve endings were considered to be terminations of sensory neurons of the nodose ganglion. For the rat trachea, the final proof of this assumption was obtained after supranodosal vagotomy, which interrupts only the central processes of these neurons; after this, no peripheral nerve terminal degeneration did occur[29].

Comparable findings are now reported as regards the NEB. The decrease of the NC ratio and the presence of the degenerating intracorpuscular nerve endings after an infranodosal vagotomy demonstrate the vagal origin of the NEB innervation. These results alone can already be taken as evidence that the NEB innervation is mainly sensory since only such fibers run directly to the lungs without forming synapses on ganglion cells.

Any motor fibers that might run directly from the brain stem to the respiratory epithelium would be expected to degenerate after supranodosal vagotomy. This procedure does not, however, cause degeneration of the NEB intracorpuscular nerve endings. This implies that after supranodosal vagotomy the neural processes, interrupted by infranodosal vagotomy, remain in contact with their cell bodies, which must therefore be localized in the nodose ganglion.

In conclusion our results can be conveniently explained by assuming that most of the NEB intracorpuscular nerve endings are terminals of neuronal processes whose cell bodies are located in the nodose ganglion of the vagus nerve. Since these neurons are sensory, an important new neuroanatomical argument has been found which favors the neuroreceptor nature of the NEB.

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