EVIDENCE FOR NEUROEPITHELIAL BODIES (NEBs) AS MECHANO-
TRANSDUCERS IN THE INTRAPULMONARY AIRWAY EPITHELIUM: A
MULTILABEL IMMUNOHISTOCHEMICAL AND LIVE CELL IMAGING STUDY

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Neuroepithelial bodies (NEBs) are morphologically well-defined airway receptors, composed of densely innervated groups of neuroendocrine cells that are shielded from the airway lumen by Clara-like cells, and together form the so-called NEB microenvironment. Because NEBs receive at least two different populations of myelinated vagal afferents (one of them expressing P2X\textsubscript{2/3} ATP receptors) with intraepithelial nerve terminals, we suggest that NEBs are the morphological counterparts of at least a subpopulation of the electrophysiologically characterized vagal mechanosensory airway receptors. This study was designed to find evidence for NEBs as airway mechanotransducers, by combining high resolution confocal live cell Ca\textsuperscript{2+} imaging and multilabel immunohistochemistry.

Hypo-osmotic solutions cause cell swelling that will increase pressure in the tight junction-sealed airway epithelium, and more specifically in the NEB microenvironment. Hypo-osmotic stimulation may, therefore, be an interesting approach to study mechanosensitivity in the NEB microenvironment. Mouse lung vibratome slices (120 µm thick) were used for confocal live cell imaging of pulmonary NEBs in an imaging chamber perfused with a physiological solution (290 mOsm/ kg H\textsubscript{2}O). In these slices, we are able to visualize NEBs by loading with the styryl pyridinium dye 4-Di-2-ASP, and Ca\textsuperscript{2+}-mediated cell activation by loading with the intracellular Ca\textsuperscript{2+} indicator Fluo-4. Short term (30s) stimulation of the lung slices with a hypo-osmotic solution (230 mOsm/ kg H\textsubscript{2}O) resulted in a fast, reversible and reproducible Ca\textsuperscript{2+} rise in NEB cells. Osmomechanical activation of NEB cells gives rise to a typical delayed activation of surrounding Clara-like cells, mediated by the release of ATP [1].

Because the osmomechanical activation of NEB cells seems to be dependent on extracellular Ca\textsuperscript{2+}, we investigated the expression of Ca\textsuperscript{2+} permeable osmo- and mechanosensitive TRP channels on NEB cells. Immunofluorescence of mouse lung cryosections showed the expression of TRPC5, a Ca\textsuperscript{2+} permeable hypo-osmotically and mechanically gated channel, on the apical membrane of NEB cells. The hypo-osmotic activation of NEB cells in lung slices was prevented by GsMTx-4 (5 µM), a toxin that blocks TRPC5. Gadolinium (500 µM), reported to activate TRPC5 channels, evoked a Ca\textsuperscript{2+} rise in NEB cells.

In conclusion, NEB cells revealed a pathway for hypo-osmotic/mechanical activation via TRPC5, resulting in a Ca\textsuperscript{2+} rise and exocytosis of ATP. These data confirm the potential role of NEBs as airway mechanotransducers, able to activate the NEB-associated P2X\textsubscript{2/3} ATP receptor expressing myelinated vagal afferents upon mechanical stimulation.

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