4-Di-2-ASP, SELECTIVE MARKER OF NEUROEPITHELIAL BODIES IN LIVING LUNG SLICES OF DIFFERENT ANIMAL SPECIES

Isabel Pintelon, Ian De Proost, Inge Brouns, Hans Van Herck , Jeroen Van Genechten, Frans Van Meir, Jean-Pierre Timmermans, Dirk Adriaensen
Laboratory of Cell Biology and Histology,
University of Antwerp
Groenenborgerlaan 171, B-2020 Antwerp, Belgium
e-mail: isabel.pintelon@ua.ac.be

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Pulmonary neuroepithelial bodies (NEBs), are innervated groups of neuroendocrine cells, located in the epithelium of intrapulmonary airways in many animal species. Our present understanding of the morphology of NEBs is comprehensive, but direct physiological studies have so far been sparse because of their extremely diffuse distribution, making NEBs inaccessible *in vivo*, and by the lack of a reliable *in vitro* model. The aim of this study was to optimise an *in vitro* method for live cell imaging based on 200-300µm vibratome slices of living lungs, a model that encloses NEBs and at least part of their complex innervation.

Styryl pyridinium dyes were selected as plausible candidates for the vital staining of NEBs in fresh lung slices. Especially 4-(4-diethylaminostyryl)-N-methylpyridinium iodide (4-Di-2-ASP), which was known as a vital stain for nerves and appeared to specifically label neuroendocrine cells in the skin, looked promising.

4-Di-2-ASP showed brightly fluorescent cell groups in the airway epithelium of living lung slices. In addition, nerve fibres also seemed to be labelled. The location and morphology of the 4-Di-2-ASP-stained intraepithelial cell groups were reminiscent of the presence of NEBs. To prove the latter, 4-Di-2-ASP-fluorescent cell groups were imaged in a confocal microscope, and subsequently the lung slices were fixed and immunostained for protein gene-product 9.5 (PGP9.5), a general marker for neuronal and neuroendocrine cells. After PGP9.5 immunostaining, the 4-Di-2-ASP-labelled cell groups in the epithelium appeared to unequivocally coincide with the presence of pulmonary NEBs. 4-Di-2-ASP fluorescence generally displayed a granular staining pattern in the labelled NEB cells, which remained detectable for more than 24 hours. Moreover, time-lapse movies recorded with the dual spinning disk confocal Ultra*VIEW* live cell imager (PerkinElmer) revealed that the fluorescent granules represented cell organelles that made rapid movements in the cytoplasm of the labelled cells. Even up to 24 hours after 4-Di-2-ASP labelling, these movements could be used to easily evaluate that NEB cells in the lung slices were viable and active.

The presented *in vitro* model and 4-Di-2-ASP staining procedure for pulmonary NEBs appeared to be equally reproducible in the lungs of diverse animal species that are important for NEB research e.g. rat, mouse, hamster and rabbit. Finally, diverse immunocytochemical procedures were shown to be applicable on the lung slices.

The clear and easy visualisation of intact living NEBs in an *in vitro* lung slice model offers great opportunities to directly monitor and manipulate NEB cells and their complex innervation in a fluorescent live cell imaging set-up. Since the reported 4-Di-2-ASP staining of NEBs appears to be highly reliable and reproducible for lung slices of several animal species, no more limitations hamper the selection of an animal model. Moreover, an integrated approach which combines physiological and functional morphological studies opens additional possibilities for elucidating the function(s) of pulmonary NEBs.

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