

# **Immunofluorescence Investigations on Neuroendocrine Secretory Protein 55 (NESP55) in Nervous Tissues**

AKADEMISK AVHANDLING

som för avläggande av medicine doktorsexamen vid Sahlgrenska akademien vid Göteborgs universitet kommer att offentligens försvaras i föreläsningssal Arvid Carlsson, Medicinargatan 3A, Göteborg, fredagen den 18 April, klockan 13.00

av

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## **Avhandlingen baseras på följande delarbeten:**

- I. **Yongling Li**, Linda Xiu-e Hou, Annika Aktiv and Annica Dahlström (2005). Immunohistochemical characterization of differentiated CAD cells: expression of peptides and chromogranins. *Histochem Cell Biol* 124(1): 25-33.
- II. **Yongling Li**, Zhanyou Wang and Annica Dahlström (2007). Neuroendocrine secretory protein 55 (NESP55) immunoreactivity in male and female rat superior cervical ganglion and other sympathetic ganglia. *Auton Neurosci* 132(1-2): 52-62.
- III. **Yongling Li** and Annica Dahlström. Peripheral projections of NESP55 containing neurons in rat sympathetic ganglia. *Auton Neurosci* (Accepted).
- IV. **Yongling Li**, Reiner Fischer-Colbrie and Annica Dahlström (2008). Neuroendocrine secretory protein 55 (NESP55) in the spinal cord of rat: An immunocytochemical study. *J Comp Neurol* 506(4): 733-44.

# **Immunofluorescence Investigations on Neuroendocrine Secretory Protein 55 (NESP55) in nervous tissues**

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## **ABSTRACT**

The chromogranin family is a group of acidic, soluble, and heat-stable proteins widespread in various neuronal, neuroendocrine and endocrine tissues, where they are subcellularly located in the secretory granules, participating in the formation of the granules. Extracellularly, chromogranins may act as protein precursors, proteolytically processed to various small bioactive peptides. Neuroendocrine secretory protein 55 (NESP55) is the most recently identified member of the chromogranin family. It is structurally related to other chromogranins. However, the biological similarity between NESP55 and its siblings has not been firmly established yet, and knowledge about NESP55 is still limited compared with other chromogranins. In the present study, we focused on the distribution and localization of NESP55 in a number of neuronal tissues using immunohistochemistry. Furthermore, the peripheral projections of NESP55 containing sympathetic postganglionic cells were investigated.

In the CNS-derived CAD cell line, NESP55, like other peptides/chromogranins, was expressed in the cell body and the long processes in a granular pattern. In addition, NESP55-IR was distinctly observed in fringe-like short processes around the cell body and along the long processes. GAP43-IR, a protein highly associated with outgrowth of neurites and development, partially overlapped with NESP55-IR in this structure. In the autonomic nervous system, NESP55 was expressed in a subpopulation of the principal neurons in all rat sympathetic ganglia studied. In the SCG, NESP55 containing neurons were found to project to the submandibular gland, the cervical lymph nodes, the iris, and the forehead skin. Some of these target-projecting neurons contained also NPY-IR, a peptide with vasoconstriction effects. The NESP55 containing SG neurons were observed to project to the forepaw pad. Among these paw pad-projecting neurons, a subpopulation contained CGRP-IR (a peptide with sudomotor effects). A subpopulation, which expressed NPY-IR, was also observed. In the rat spinal cord, NESP55-IR was found in various spinal neurons throughout the lamina IV-X, including motoneurons, autonomic sympathetic/parasympathetic neurons, interneurons and the LSN. Many of these NESP55 containing neurons were also immunoreactive to ChAT, a cholinergic marker. The lamina I-III and the sensory dorsal root ganglion lacked NESP55-IR.

The intracellular distribution of NESP55-IR in the spinal motoneurons appeared different from that in the sympathetic neurons. In the spinal motoneurons, NESP55-IR, with an appearance of dust-like particles, was observed diffusely present in the whole cytoplasm; in contrast, in the sympathetic neurons, NESP55-IR appeared to be stored in large granules, restricted to the perinuclear region of the ganglionic cells, and overlapping with the Golgi marker, TGN38.

In conclusion, the present study demonstrated that NESP55 was expressed in different functional groups of neurons in the rat sympathetic ganglia and in the spinal cord. The expression of NESP55 in the CAD cells was exceptional. Our findings may add information about this novel protein and further our understanding of its functional significance. Moreover, the finding of the striking difference in the intracellular distribution of NESP55-IR in motoneurons versus autonomic neurons supports the previous suggestion that NESP55 may be involved in both constitutive and regulated secretory pathways.

**Keywords:** chromogranins, neuropeptides, secretory pathway, the CAD cell line, rat, spinal cord, sympathetic ganglia, retrograde tracing, confocal microscopy.

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