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Autonomic Neuroscience: Basic and Clinical 141 (2008) 1 – 9

AUTONOMIC NEUROSCIENCE: Basic & Clinical

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Peripheral projections of NESP55 containing neurons in the rat sympathetic ganglia

Yongling Li*, Annica Dahlström

Department of Anatomy and Cell Biology, Institute of Biomedicine, Göteborg University, Box 420, SE-405 30 Göteborg, Sweden

Received 4 December 2007; received in revised form 7 March 2008; accepted 20 March 2008

Abstract

The peripheral projections of neurons expressing neuroendocrine secretory protein 55 (NESP55), a novel member of the chromogranin family, were studied by retrograde tracing technique. It was found that NESP55 positive neurons in the rat superior cervical ganglion projected to a number of targets including the submandibular gland, the cervical lymph nodes, the forehead skin, the iris, but not to the thyroid. Among these NESP55 positive target-projecting neurons, a subpopulation contained neuropeptide Y (NPY), a vasoconstrictor. Forepaw pad projecting neurons were found exclusively in the stellate ganglion, almost all of which (approximately 90%) were immunoreactive to NESP55. Colocalization of NESP55 and calcitonin gene-related peptide (CGRP), a peptide involved in sudomotor effects, was observed in a subpopulation of these paw pad projecting neurons, as was colocalization of NESP55 and NPY. The data suggest that NESP55 may have a functional role in some populations of sympathetic neurons.

Keywords: Retrograde tracing; Chromogranins; Peptides; ANS; Chemical coding

1. Introduction

Postganglionic sympathetic neurons show two main phenotypes characterized by their contents of the classical neurotransmitters: noradrenaline and acetylcholine. The majority, expressing noradrenline, are noradrenergic neurons responsible for autonomic functions including vasoconstrictor, pilomotor, secretomotor, and visceromotor activities, etc. (Gibbins, 1995). A much smaller population, for instance, those projecting to the paw sweat glands and the rib periosteum is cholinergic, utilizing acetylcholine as the transmitter (Anderson et al., 2006). In addition, a wide variety of neuropeptides has been observed in the sympathetic postganglionic neurons (Gibbins, 1992; Forehand, 1995; Gibbins, 1995; Grkovic and Anderson, 1997; Bergner et al., 2000). Distinctive combinations of these neurochemicals in the postganglionic neurons, together with the preganglionic inputs, chemically code target-specific pathways (Landis and Fredieu, 1986; Anderson et al., 1995; Grkovic and Anderson, 1997; Chanthaphavong et al., 2003; Murphy et al., 2003; Richardson et al., 2006). The submandibular salivary gland of the rat was found to be innervated by neuropeptide Y (NPY) immunonegative postganglionic neurons, which, however, received calretinin positive preganglionic innervation (Grkovic and Anderson, 1995); Cholinergic sudomotor neurons contained immunoreactivities (IR) for vasoactive intestinal peptide (VIP) and calcitonin gene-related peptide (CGRP) but were always devoid of calbindin-IR. However, cholinergic neurons projecting to the periosteum contained VIP-IR and sometimes calbindin-IR, but always lacked CGRP-IR (Hohmann et al., 1986; Anderson et al., 2006). Chemical coding of the postganglionic neurons differs among species. Opioid peptides were found in the noradrenergic NPY positive vasoconstrictor neurons of guinea pigs, rats, and cattle, but not in mice, nor in humans (Gibbins, 1995). Cholinergic vasodilator neurons were observed in cats (Anderson et al., 1995), and guinea pigs

* Corresponding author. Department of Anatomy and Cell Biology, Box 420, SE-405 30 Göteborg, Sweden. Tel.: +46 31 786 3366; fax: +46 31 82 96 90.
E-mail address: yongling.li@anatcell.gu.se (Y. Li).

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doi:10.1016/j.autneu.2008.03.008

(Anderson et al., 1996), but never in rats (Guidry and Landis, 2000).

The chromogranin family is a group of acidic, soluble, and heat-stable proteins widespread in various neuronal, neuroendocrine and endocrine tissues, where they were suggested to have both intracellular and extracellular roles, acting as inducers in the formation of the secretory granules or as small peptide precursors, respectively (Taupenot et al., 2003). Neuroendocrine secretory protein 55 (NESP55) is the most recently identified member of this family (Ischia et al., 1997). It, like its siblings, is subcellularly located in the secretory granules or the large dense core vesicles of the adrenal medulla and the splenic nerves (Ischia et al., 1997; Bauer et al., 1999b). However, NESP55 was also observed in vesicles with a density slightly lighter than the large dense core vesicles and constitutively secreted into the exterior in the mouse AtT-20 cells (Eder et al., 2004). In the rat spinal cord, NESP55-IR was found to be concentrated in the prenuclear region in the sympathetic neurons. In contrast, in the spinal motoneurons, NESP55-IR was diffusely distributed throughout the whole cytoplasm (Li et al., 2008). Thus, NESP55 may be involved in both regulated and constitutive pathways. NESP55 is proteolytically processed to smaller peptide, like GAIPIPRRH, in various bovine tissues to a varying degree (Lovisetti-Scamihorn et al., 1999). Moreover, NESP55 is genomically imprinted and expressed exclusively from the maternal allele (Hayward et al., 1998; Plagge et al., 2005).

Previously, we demonstrated that NESP55 was expressed in both noradenergic and cholinergic postganglionic neurons of the rat (Li et al., 2007). In the present study, we attempted to analyze the peripheral projections of these NESP55 positive sympathetic neurons by retrograde tracing technique. Our results showed that the majority of the forepaw pad projecting neurons contained NESP55-IR. Also, NESP55 positive neurons in the rat superior cervical ganglion (SCG) were shown to innervate a number of target tissues.

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley rats ((9–10 weeks old) purchased from B & K Universal (Aldbrough, England) were used in this study. The animals were housed on a 12 h light/dark cycle with food and water available *ad libitum*. All experimental procedures were approved by the Animal Ethical Committee of Gothenburg University and all efforts were made to minimize animal suffering and the number of animal used.

2.2. Retrograde tracer injections and tissue preparation

Fluoro-Gold (fluorochrome, Englewood, USA), dissolved as 4% in distilled water, was used as tracer to examine the peripheral projections of NESP55 positive neurons in different sympathetic ganglia (Schmued and Fallon, 1986). Under sodium pentobarbital (50 mg/kg, i.p.) anesthesia, 4% Fluoro-Gold was injected unilaterally (right side) into five different targets including the submandibular gland, the thyroid, the cervical lymph nodes, the forepaw pad and the anterior chamber of the eye (iris). The forehead skin (around midline area) was also applied as a target. For each investigated target, three animals were used. The submandibular salivary gland, the thyroid and the lymph nodes were exposed via a midline neck incision. Injections of Fluoro-Gold (1–1.5 μl each) were made into the above organs at 2–5 sites and the skin incisions were sutured. Tracer injections, 3–4, were also done for the forehead skin. In the forepaw 1–2 injections of tracer were made into each paw pad tubercle, totally up to 10 sites. A total volume of 4 μl of tracer was injected into the anterior chamber of the eye after insertion of the needle into the lateral corner of the anterior chamber. In all cases, the injecting device, Microliter Syringe (Hamilton Bonaduz, Schweiz), was left in the place for 1–2 min after injection to minimize dye leakage. Any leakage after this time was soaked up with a cotton pad.

After a survival period of 4–5 days, all animals were perfused transcardially with 4% paraformaldehyde (pH 7.4). The SCG, the stellate ganglion (SG), as well as the sympathetic chain ganglia from both sides, were then removed and post-fixed overnight in the same fixative and stored at 4 °C in a PBS solution containing 0.1% sodium azide and 20% sucrose. The ganglia were frozen with compressed CO_2, sectioned longitudinally in a cryostat at 10–12 μm, and mounted on gelatinized glass slides for immunohistochemistry.

2.3. Immunofluorescence procedures

2.3.1. Primary antibodies

Polyclonal Guinea pig anti-NESP55, kindly donated by Dr. Reiner Fischer-Colbrie, University of Innsbruck, Austria. The antiserum was prepared against a synthetic octapeptide representing the C-terminus (GAIPIRRH, amino acids 234–241) of NESP55 and staining a single band of Mr 55,000 on western blots (Ischia et al., 1997), dilution 1:4000. In brains of NESP55 knock-out mice (Plagge et al., 2005) no positive staining was seen with this antiserum (Ischia et al., 1997; Eder et al., 2004).

Polyclonal rabbit anti-calcitonin gene-related peptide (CGRP), produced against a synthetic peptide corresponding to amino acids 1–37 of rat CGRP (Cambridge Research Biochemicals, Cleveland, UK; cat# CA-08-220), dilution 1:400.

Polyclonal rabbit anti-neuropeptide Y (NPY), produced against synthetic porcine NPY-KLH (Sigma, St. Louis, MO, USA; cat# N9528), dilution 1:4000.

Polyclonal rabbit anti-Tyrosine Hydroxylase (TH), produced against tyrosine hydroxylase purified from pheochromocytoma (Sigma-Aldrich, Sweden; cat# T8700), dilution 1:800

Polyclonal goat anti-neuropeptide Y (NPY), produced against synthetic porcine neuropeptide tyrosine (Affiniti