Polish Journal of Veterinary Sciences Vol. 25, No. 4 (2022), 617–619

DOI 10.24425/pjvs.2022.142037

Short communication

Dispersal and immunohistochemical characteristics of neurons in the stem of the porcine vagus nerve

W. Sienkiewicz[1], M. Klimczuk[1], M. Majewska[2], J. Kaleczyc[1]

[1] Department of Animal Anatomy, Faculty of Veterinary Medicine, University of Warmia and Mazury, Oczapowskiego 13, 10-719 Olsztyn, Poland
[2] Department of Human Physiology and Pathophysiology, School of Medicine, University of Warmia and Mazury in Olsztyn, Warszawska 30, 10-082 Olsztyn, Poland

Abstract

The present study investigated the distribution and chemical properties of nerve cell bodies within the trunk of the vagus nerve in juvenile female pigs (n=4) using double-labelling immunofluorescence. The neurons appeared mostly as single cells or formed streaks of cells or small ganglia. Many of the perikarya were cholinergic (VAChT-positive; VAChT+) or adrenergic (DβH+) in nature and no SP+ or CGRP+ neurons were encountered. There were no distinct left-right differences regarding the number and chemical coding of the neuronal somata, however, these characteristics significantly varied between particular nerve segments investigated. The vagosympathetic trunks, and thoracic and abdominal segments of the vagus nerve contained on average (the numerical values represent the means for both the left and right corresponding nerve segments) 142, 236, and 111 PGP 9.5-positive neurons, respectively. Proportions of cholinergic and adrenergic neurons were as follows: 0% and 100%, 54.2% and 33.2%, and 52.8% and 35.4%, respectively. Relatively many neurons in the thoracic and abdominal segments stained also for NOS (39.2% and 39.9%, respectively). It remains to be determined whether the porcine intravagal neurons represent a developmental relic, or whether they have any specific functional significance.

Key words: vagus nerve, intravagal neurons, neurotransmitter markers, immunohistochemistry, pig

Introduction

The literature in the field contains few papers reporting the presence of single neurons or microganglia within the trunk and branches of the vagus nerve in some mammalian species including humans and pigs (Botar et al. 1950, Szteyn 1969, Matysek et al. 2003). However, such essential information as that on the chemical coding of these nerve cells and its potential segmental variations is still unavailable. Therefore, the present study investigated the distribution and chemical properties of nerve cell bodies within the trunk of the vagus nerve in juvenile female pigs.

Correspondence to: W. Sienkiewicz, e-mail: sienio@uwm.edu.pl

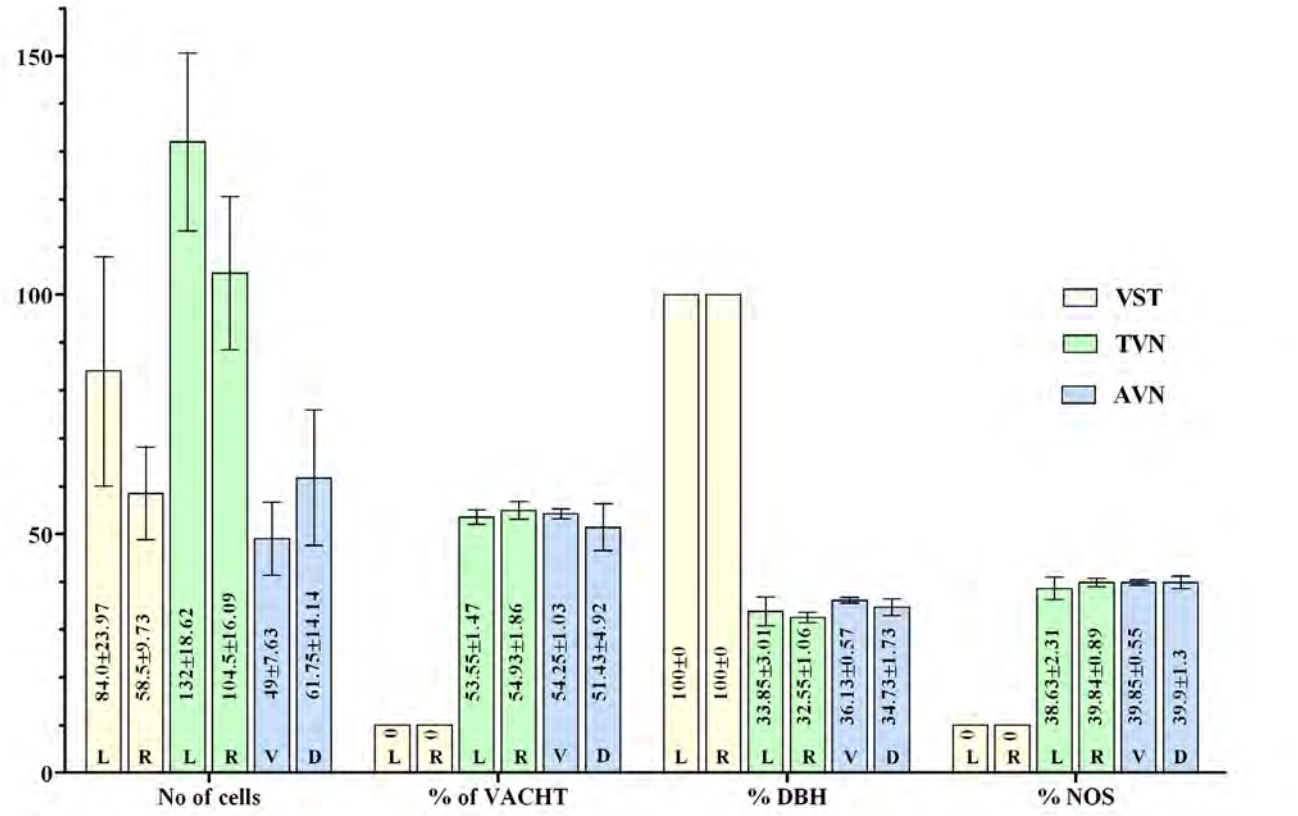

Fig. 1. Graphs of the number and chemical coding of neurons in the vagosympathetic trunk (VST) as well as the thoracic (TVN) and abdominal (AVN) segments of the left (L) and right (R) or ventral (V) and dorsal (D) vagus nerves in pigs. Bars and error bars represent means and SEM, respectively. Numerical data are given within the bars.

Materials and Methods

The study was carried out on 4 juvenile female pigs of the Large White Polish race (12-15 kg of body weight). The animals were housed and treated in accordance with the rules approved by the local Ethics Commission. The pigs were pharmacologically euthanized with pentobarbital sodium overdose, transcardially perfused with buffered paraformaldehyde (pH 7.4), and both left and right vagosympathetic trunks as well as the complete thoracic and abdominal segments of the vagus nerve were collected. The cryostat, longitudinal sections of the nerves were processed for double-labelling immunofluorescence (for the method and controls of staining specificity, see Sienkiewicz et al. 2010) using antibodies (for details, see Kaleczyc et al. 2020) against protein gene product 9.5 (PGP 9.5; to visualize the nerve cell bodies), vesicular acetylcholine transporter (VAChT), dopamine β-hydroxylase (DβH), substance P (SP), calcitonin gene-related peptide (CGRP) and nitric oxide synthase (NOS).

The sections labelled were viewed under Zeiss Axiophot microscope equipped with epi-fluorescence and an appropriate filter set. They were also investigated and images were recorded with a Zeiss LSM 700 confocal laser scanning microscope (Zeiss, Jena, Germany), using a 20 × 0.8 plan-apochromat objective lens and ZEN Software 2009. Channels were scanned consecutively to avoid cross-talk.

To determine percentages of the neuronal subpopulations, at least 100 PGP 9.5-positive neuronal profiles for each combination of antisera were analysed in one animal. To avoid double-counting of the same neurons, appropriate distance (minimum 5 sections = 50 μm) between the sections was maintained. The number of immunolabelled profiles was calculated as a percentage of the immunoreactive neurons in relation to all PGP-9.5-positive perikarya counted.

Results and Discussion

The left and right vagus nerves contained many PGP 9.5-positive neurons (Fig. 1). They occurred as solitary nerve cell bodies or formed streaks of neurons or small ganglia. The numerous perikarya were cholinergic (VAChT-positive; VAChT+) or adrenergic (DβH+) in nature and many neuronal somata stained for NOS but no SP+ or CGRP+ nerve cells were encountered (Fig. 2A-C). There were no distinct left-right differences regarding the number and chemical coding of the neurons, however, these characteris-

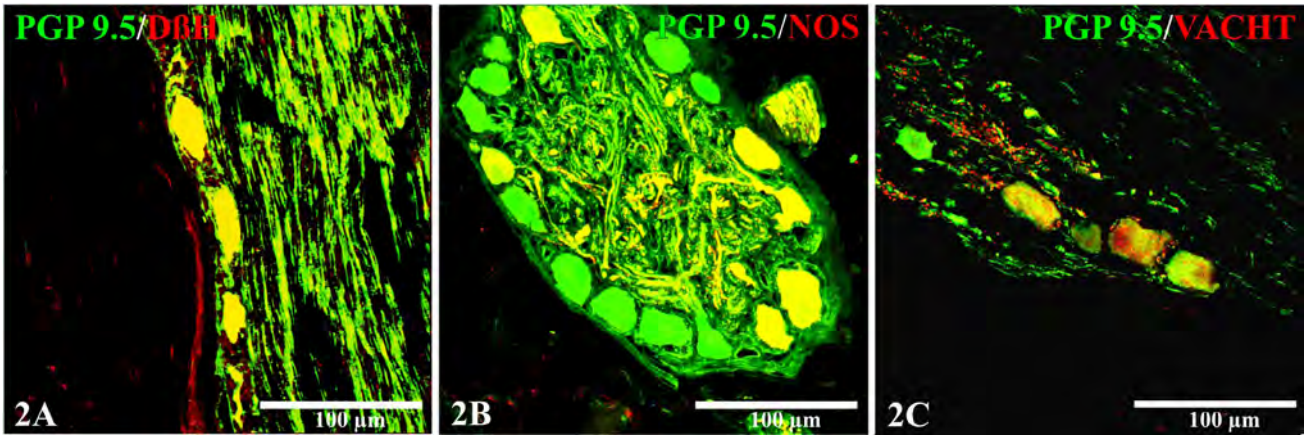

Fig. 2. Confocal laser scanning microscope images showing the distribution of protein gene product 9.5-positive [PGP 9.5+; green; Alexa 488 visualization (A488)] and DβH+ (Fig. 2A), NOS+ (Fig. 2B) or VAChT+ (Fig. 2C) [red; Alexa 555 visualization (A555)] neurons in sections from the left VST, right TVN and ventral AVN, respectively; green and red channels were digitally superimposed, so neurons that stained for both PGP and one of the other markers are yellow to orange.

tics significantly varied between particular nerve segments investigated (Figs. 1-4).

The present study revealed that relatively many neurons are distributed within the trunk of the porcine vagus nerve. This neuronal population comprises not only cholinergic but, unexpectedly, many adrenergic and also non-adrenergic, non-cholinergic nerve cells.

It is difficult to speculate about the reason for the presence of the neurons in a rather unusual location such as the trunk of the vagus nerve. Nevertheless, the very sparse literature on the subject contains some information which gives rise to certain assumptions. It seems that this may be related to the phenomena concerning the ontogenetic development of the peripheral autonomic nervous system (for reviews see Young et al. 2011, Uesaka et al. 2016). It has been established on the basis of studies conducted mainly on laboratory mammal and chick embryos that enteric neuronal precursors that derive from the vagal (caudal hindbrain) neural crest cells (NCC) colonize the foregut while the sacral NCC-originated precursors invade the hindgut. Moreover, migrating vagal NCC and vagal nerve fibres follow the same pathway from the hindbrain to the foregut, but the cells exit the hindbrain and arrive at the gut prior to the nerve terminals. Sympathetic ganglia were shown to arise from NCC that emigrate from the neural axis caudal to the fifth somite. Thoracic neural crest derived cells and some those that emigrate from the neural tube adjacent to somites 1–4 (anterior vagal) were proposed to give rise to the superior cervical ganglion (SCG). Furthermore, the trunk NCC are the origin of paravertebral sympathetic ganglia. Thus this fairly ordered developmental system may correspond in some way with a rather distinct distribution pattern of specifically coded neurons in different parts of the porcine vagal trunk; only adrenergic neurons are present in the vagosympathetic trunk, while in the remaining vagal segments, adrenergic neurons accounted for about a third and cholinergic neurons for more than half of all nerve cells. However, it remains to be determined whether these intravagal neurons represent a developmental relic, or whether they have any specific functional significance.

References

Botar J, Afra D, Moritz P, Schiffmann E, Schole M (**1950**) Die Nervenzellen und Ganglien des N. vagus. Acta Anat 10: 284-314.

Kaleczyc J, Kasica-Jarosz N, Pidsudko Z, Dudek A, Klimczuk M, Sienkiewicz W (**2020**) Effect of castration on pelvic neurons in the male pig. Histochem Cell Biol 153: 135-151.

Matysek M, Krakowska I, Boratyński Z, Łuszczewska-Sierakowska I (**2003**) Ganglionic cells in the vagus nerve trunk in the domestic pig. Medicina Veterinaria 2 (1): 27-31.

Sienkiewicz W, Dudek A, Kaleczyc J, Chrószcz A (**2010**) Immunohistochemical characterization of neurones in the hypoglossal nucleus of the pig. Anat Histol Embryol 39: 152+159.

Szteyn S (**1969**) Komórki zwojowe w pniu nerwu błędnego owcy. Annales UMCS Lublin-Polonia.

Uesaka T, Young HM, Pachnis V, Enomoto H (**2016**) Development of the intrinsic and extrinsic innervation of the gut. Dev Biol 417 (2): 158-167.

Young HM, Cane KN, Anderson CR (**2011**) Development of the autonomic nervous system: a comparative view. Auton Neurosci 165: 10-27.