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Substance P and Calcitonin Gene-Related Peptide Containing Neurons in the Feline Spinal and Superior Mesenteric Ganglia Projecting to the Distal Ileum

I. Stoyanova, N. Lazarov

Department of Anatomy, Faculty of Medicine, Thracian University, Stara Zagora

Combined retrograde tracing with the fluorescent tracer Fast Blue (FB) and immunofluorescent cytochemical methods were used to study the distribution and neurochemical identification of the neurons innervating the distal part of the ileum in cats. As revealed by retrograde tracing, FB-positive neurons projecting to the small intestine were located in the prevertebral superior mesenteric ganglion (SMG) and the dorsal root ganglia (DRG) at the level Th_{7-12}. In the SMG, the majority of the projecting neurons resided in the upper part of the ganglion, suggesting a somatotopic organization within the ganglion. Immunocytochemistry revealed two populations of retrogradely labelled neurons: substance P (SP)- and calcitonin gene-related peptide (CGRP)-positive. We concluded that the SMG and DRG Th_{7-12} should be considered as a prominent source of SP- and CGRP-immunoreactive sympathetic and sensory projections to the distal ileum of the cat.

Key words: superior mesenteric ganglion, dorsal root ganglia, retrograde tracing, immunohistochemistry, cat.

Introduction

The innervation of the distal ileum was studied in different animals. The sympathetic supply originates from the prevertebral ganglia: the coeliac ganglion provides the major input to the proximal gut regions, while the distal gut receives input from the superior and inferior mesenteric and hypogastric ganglia. The parasympathetic and sensory neuronal perikarya projecting to this part of the gut are located in the vagal dorsal motor nucleus and in the nodose ganglion and in dorsal root ganglia (DRG) [1], respectively.

Apart from the classical neurotransmitters acetylcholine and noradrenalin, a large number of the neurons in the sympathic ganglia contain small biologically active peptides, which act as neurotransmitters or neuromodulators in the autonomic nervous system: substance P, somatostatin, NPY, VIP, and CGRP [2]. The distribution of some of these transmitters through a feline SMG was described by Stoyanova et al. [3].

Although some peptidergic pathways to the gastrointestinal tract have been identified, in certain species our knowledge about the origin and projections of all

these fibres is still incomplete and some of the data are contradictory. The present work was undertaken to determine the distribution and neuropeptide contents of the neurons and neuronal fibres in feline SMG and DRG, projecting to the distal part of the small intestine.

Material and Methods

Five adult cats were anesthetized and a solution of 2% retrograde neuronal tracer FB was injected into the wall of the distal ileum. The animals were perfused after 35-40 days, with 2 L 4% paraformaldehyde and 0.2% picric acid in 0.1 M phosphate buffered saline. The SMG and DRG Th_6-L_2 were removed, postfixed, frozen and 20 μm thick sections were cut on a cryostat at –20°C. The indirect immunofluorescent technique was applied with primary antibodies, rabbit anti-SP- or CGRP-antiserum and secondary antibody donkey-anti-rabbit IgG conjugated to fluorescein isothiocyanate.

Results

In the SMG the tracer was found in neurons located predominantly in the upper part of the ganglion (Fig. 1). Two types of FB-labelled neurons were differentiated: magnocellular multipolar ganglionic cells, which were often clustered and a second group of parvocellular neurons with a less pronounced multipolar shape. A fairly high number of the FB-marked cells were CGRP-immunoreactive (Fig. 2 — *a*, *b*). In addition, numerous FB/CGRP-containing varicose neuronal fibres were found surrounding CGRP-negative magnocellular ganglionic cells. Less parvocellular SMG neurons projecting to the ileum were SP-positive. However, more often FB/SP-IR neuronal fibres were detected. In the investigated spinal ganglia FB-labelled primary sensory neurons were found at levels $Th_{7\text{-}12}$. Relatively large proportions of them were SP- or CGRP-positive.

Fig. 1. A large number of FB-labelled neurons in the upper part of the SMG (× 50)

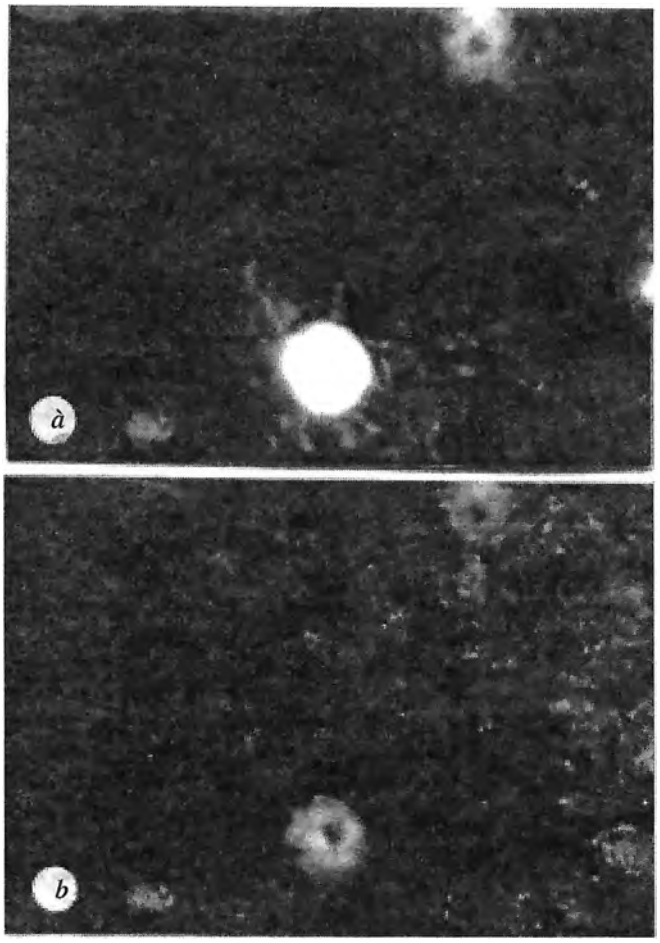

Fig. 2. Double labelling of parvocellular SMG projecting neurons — *a* FB; *b* CGRP (× 250)

Discussion

The present work reveals that the distal ileum in the cat receives both autonomic and sensory innervation. As already reported in a previous study [4], an organotopic arrangement of nerve cell bodies within the SMG is present: those innervating the distal part of the ileum are located in the upper part of the ganglion.

It was found that in the rat the primary afferents projecting to the ileum are located in DRG $Th_{10\text{-}13}$ [5], whereas in the cat we detected FB-labelled cells at level $Th_{7\text{-}12}$. CGRP is one of the major neuropeptides, expressed in the sensory relaying regions of the nervous system. CGRP is generally co-localized with SP in the primary afferent nociceptors, and both neuropeptides play a role in mediating visceral nociceptive transmission [6].

We concluded that the SMG and DRG $Th_{7\text{-}12}$ should be considered as a prominent source of SP- and CGRP-immunoreactive sympathetic and sensory projections to the distal ileum of the cat. Most of the SMG and DRG projecting neurons are CGRPergic, and a few of these are SPergic.

References

1. Pidsudko, Z. et al. The influence of inflammation on the expression of neuropeptides in the ileum-projecting primary sensory neurons in the pig. — Folia Morphol. (Warsz.), **62**, 2003, 235-237.
2. Hokfelt, T. et al. On the occurrence of substance P-containing fibres in sympathetic ganglia: immunohistochemical evidence. — Brain Res., **132**, 1977, 29-41.
3. Stoyanova, I., D. W. Scheuermann, C. Chouchkov. Neurochemical features of feline superior mesenteric ganglion. — It. J. Anat. Embryol., **103**, 1998, 35-43.
4. Stoyanova, I., C. Chouchkov, D. W. Scheuermann. Immunocytochemical localization of the neurons in the superior mesenteric ganglion innervating the small intestine of the cat. — Ann. Anat., **179**, 1997, 517-523.
5. Cervero, F., K. A. Sharkey. An electrophysiological study of intestinal afferent fibres in the rat. — J. Physiol., **401**,1988, 381-397.
6. Levin, J. D., H. L. Fields, A. I. Basbaum. Peptides and the primary afferent nociceptor. — J. Neurosci., **13**, 1993, 2273-2286.