

Spinal projections of medium sized sensory neurons that express Calcitonin Gene-Related Peptide but not Substance P in mice

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Many small diameter sensory neurons in dorsal root ganglia (DRG) contain both calcitonin gene-related peptide (CGRP) and substance P (SP). These neurons generally have a nociceptive function. However, in DRG of mice, a population of medium diameter neurons express CGRP but not SP. The projections and functions of these neurons are not known. Therefore, we have combined *in vitro* axonal tracing with multiple-labelling immunohistochemistry to map the projections of these neurons to the cervical spinal cord.

Mice (C57/B16) were anaesthetised with inhaled isoflurane and exsanguination, prior to removal of the upper spinal cord with intact brachial plexus and dorsal root ganglia. Neurobiotin (NB) was applied to the C7 ventral ramus and the brachial plexus-DRG-spinal cord preparation was incubated for 4 hours. NB was subsequently detected with streptavidin-DTAF in spinal cord and DRG sections, which were also labelled for CGRP and SP.

The majority of the DRG neurons were filled with NB. Approximately one-third of the sensory neurons in C7 DRG expressed CGRP. 44% of these neurons did not contain detectable levels of SP and had an average soma size of $522 \pm 26 \mu\text{m}^2$. Within cervical spinal cord, terminals containing CGRP were prominent in the superficial dorsal horn (lamina I) and deeper dorsal horn (lamina IV). CGRP terminals lacking SP were most prominent in lateral areas of lamina I and in lamina IV. Surprisingly these terminals were only occasionally filled with NB. NB filled fibres mainly projected into the medial dorsal horn. These fibres included large myelinated fibres that extended into the ventral horn, and smaller diameter fibres that penetrated lamina IV. Some of these small diameter fibres in lamina IV contained CGRP and the majority of these lacked SP. Small diameter fibres filled with NB and CGRP were also present in laminae I and II. A subpopulation of these fibres did not contain SP.

Although the majority of sensory neurons in the DRG are filled with NB it appears that there is an under-representation of small diameter NB filled fibres in the spinal cord. A likely explanation is that these neurons of the C7 DRG project to other spinal segments rostral-caudal to the C7 segment. Our data show that 44% of the sensory neurons positive for CGRP do not contain detectable levels of SP. Based on their multiple somatotopic projections we propose that neurons containing CGRP without SP may represent a subpopulation of polymodal mechanoreceptors.