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Rectospinal neurons: evidence for a direct projection from the enteric to the central nervous system in the rat*

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Wheat germ agglutinin–horseradish peroxidase conjugate (WGA–HRP), horseradish peroxidase (HRP), and Fluorogold injections were made into the spinal cord segments L_4–S_2, and HRP was applied to cut L_6 and S_1 dorsal roots in the rat. These procedures resulted in retrograde labeling of neuronal cell bodies in the rectal wall. Labeled neurons were found both inside and outside myenteric ganglia. Their occurrence was restricted to 5 mm proximal of the external anal sphincter. These cell bodies might represent an additional type of afferent neuron, furnishing a direct information pathway from the rectal wall to the spinal cord.

Primary afferent neurons of the dorsal root ganglia (DRG) are known to reach the mucosa and muscular layers of the alimentary tract with their peripheral processes, whereas the central axons project to the spinal cord. Further, in recent years it has been shown by morphological and physiological techniques that sensory neurons lying in the myenteric and submucosal plexuses of the large and small intestine project centripetally [7, 13] either to the inferior mesenteric [4, 16] or to the celiac ganglia [3, 7, 18]. Occasionally, the question has been raised whether sensory neurons projecting directly to the spinal cord without intercalation might exist as well [2, 7, 12, 19]. The aim of the present study was to examine this question with respect to the innervation of the rectum.

Nine albino rats (strain ZUR:SIV, Institute of Laboratory Animal Science, University of Zürich, b.wt. 250–300 g) were deeply anesthetized with a combination of Sedalande (Delalande, 1 mg/100 g), Fentanyl (Janssen, 0.02 mg/100 g) and Valium (Roche, 0.25 mg/100 g).

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In 5 rats a laminectomy was performed to expose the spinal cord segments L_4–S_2. The dura was then split with the tip of a hypodermic needle and wheat germ agglutinin–horseradish peroxidase (WGA–HRP) conjugate (Sigma, 10–15 μl of a 2% solution in saline) was injected into the spinal cord of two animals. Fluorogold (Fluorochrome, 10–15 μl of a 2% solution in saline) [15], and a 20% aqueous solution of HRP (Boehringer Mannheim, Reinheitsgrad I) were injected in two rats and in one rat, respectively, with a glass pipette (o.d. 30–50 μm). In 4 animals ventral or dorsal roots of L_6 or S_1 were cut bilaterally, proximal to the DRGs (one pair in each case). HRP (cyrstals or a 30% aqueous solution) was applied to the distal ends of the cut spinal roots for 30 min. After survival times of two days in WGA–HRP and HRP experiments, and 7–10 days in Fluorogold experiments, animals were perfused through the ascending aorta with a Ringer prewash, a fixative (consisting of 1% paraformaldehyde, 1.25% glutaraldehyde in 0.1 M phosphate buffer in WGA–HRP and HRP experiments, or 4% paraformaldehyde in phosphate buffer in Fluorogold experiments), and a final rinse with phosphate buffer. Spinal ganglia from L_4 to S_2 with their corresponding spinal cord segments, pelvic ganglia, distal colon, and rectum were removed and immersed in 18% sucrose buffer overnight. Cryostat sections of 20–40 μm were reacted for HRP activity according to the tetramethylbenzidine (TMB)-method [11]. Myenteric ganglia were demonstrated with Neutral red counterstaining or with acetylcholinesterase (AChE) histochemistry [10] after diaminobenzidine (DAB)/$CoCl_2$ stabilization of the TMB reaction product [14]. Fluorogold labeling was detected on 20 μm sections under a Zeiss fluorescence microscope at an excitation wave length of 365 nm.

Sections of spinal cord segments L_4–S_2 from WGA–HRP, HRP, and Fluorogold injection experiments showed tracer infiltration throughout the whole cross-sectional area. Neurons and fibers in the DRGs were also filled well with label. The spinal nerves of L_4–S_2 showed many labeled axons, suggesting that ample tracer uptake in the spinal cord and transport beyond the DRG had taken place. Sections of the pelvic ganglia (from the experiments where WGA–HRP and HRP had been used) contained labeled fibers and varicose preganglionic terminations, as described in a recent study [1]. In none of our experiments could any transneuronally labeled cell bodies be seen in pelvic ganglia. Varicose terminals could not be found after HRP application to cut dorsal or ventral roots.

After WGA–HRP and Fluorogold injections into the spinal cord longitudinal sections of the rectum showed about 100 neuronal cell bodies lying mostly in the 'myenteric cleft' between the inner and outer muscle layers. Most of them were located in myenteric ganglia, which could clearly be recognized particularly in AChE (Fig. 1) counterstained sections, whereas others seemed to be outside of the ganglia among smooth muscle cells of both layers. A few cells were also seen in the adventitia. Most of the labeled neurons occurred singly. A grouping of up to 3 neurons was seen only on three sections (Fig. 2). Labeled neurons were also detected, although in smaller amounts, in experiments where free HRP had been applied to the spinal cord (Fig. 4) or to cut L_6 and S_1 dorsal roots; they were not seen after HRP application to cut ventral roots. Notably, the labeled neurons were scattered over a restricted area of

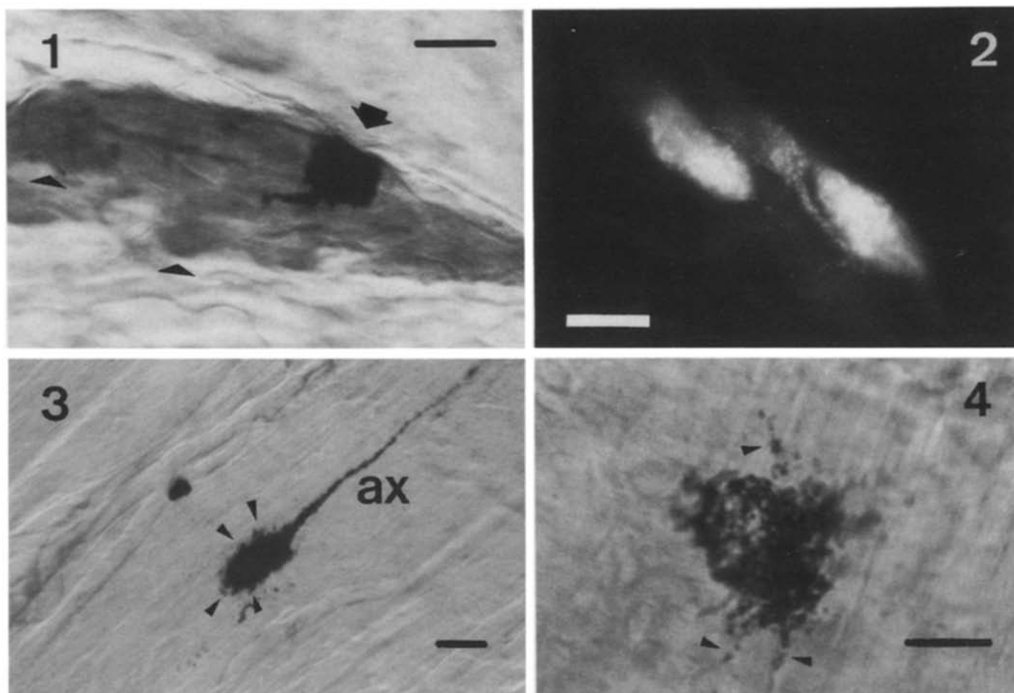

Fig. 1. Retrogradely labeled neuron (arrow) within a myenteric ganglion of the rectum after WGA-HRP tracer injection into the spinal cord. Note: AChE-positive unlabeled enteric neurons (arrowheads). Bar = 20 μm.

Fig. 2. A group of 3 labeled neurons within the rectal wall after Fluorogold injection into the spinal cord. Bar = 20 μm.

Fig. 3. Retrogradely labeled, spindle-shaped neuron within the muscular wall of the rectum after WGA-HRP injection into the spinal cord. The axon (ax) is directed orally. Note: several short processes originating from the perikaryon (arrowheads). Bar = 20 μm.

Fig. 4. Myenteric neuron within the rectum labeled by retrograde HRP transport from the spinal cord. Several thin processes (arrowheads) originate from the perikaryon. Bar = 20 μm.

5 mm proximal to the external sphincter; they neither occurred in the proximal rectum nor in the distal colon.

Regarding their shape, two different groups of cell bodies could be discerned: the majority of the cells had several short processes and were rather oval or spindle-shaped (long axis 30–60 μm) with a long, thin axon that could be traced over a distance of about 100 μm (Fig. 3). Other cells showed more stellate, multipolar contours with thin processes (Fig. 4).

Apart from these neurons, motor endplates were clearly labeled in the external anal sphincter after WGA–HRP and HRP injections into the spinal cord. Transganglionically labeled sensory fibers running between the muscle layers of the rectum, and others reaching the mucosa of the anal canal, could be seen as well. Sensory fibers were not observed in close proximity to myenteric ganglia.

The present results show that neurons located in the wall of the rectum project directly to the spinal cord. Theoretically, these neurons could have been labeled by anterograde transneuronal transport of WGA–HRP (for review see ref. 17) from preganglionic neurons. However, for a number of reasons, this is an unlikely mechanism. First, it is known that preganglionic parasympathetic neurons in the spinal cord of dogs [8] and also of rats (unpublished results) do not project to myenteric ganglia, but instead to pelvic ganglia. In our experiments no transneuronally labeled cell bodies could be detected in pelvic ganglia. Secondly, it could be assumed that transneuronal labeling had taken place by primary afferent fibers contacting myenteric ganglia. Again, this possibility is considered unlikely, as hardly any labeled sensory axons were found in the vicinity of labeled intramural neurons. Furthermore, if this possibility was the reason for the cell labeling observed, numbers of labeled cells would be expected to increase after tracer injections into the DRGs; but on the contrary, although more labeled sensory fibers were seen, the number of labeled cell bodies was considerably decreased (unpublished results) compared to spinal cord injection experiments. It should also be mentioned that HRP and Fluorogold, which produced identical labeling of intramural neurons, have not been shown to be transneuronal tracers in adult mammals (for review see refs. 15, 17). Thus, it is reasonable to assume that the cells described here were labeled by retrograde tracer transport from the spinal cord. Therefore, they represent a type of enteric afferent neuron, similar to those described in the small and large intestine [3, 4, 7, 16], but projecting beyond the prevertebral ganglia to the spinal cord. The fact that the neurons in question were only detected in experiments where dorsal roots and not where ventral roots had been cut, suggests a pathway of their axons through dorsal roots to the spinal cord.

The location of these neurons might provide clues for speculations about their function. Some of them, making contact with the mucle tissue, might act as first order mechanosensory neurons [19], somewhat reminiscent of crustacean stretch-receptors [5]. Those lying in the myenteric ganglia could serve a similar function. Additionally, they might be influenced by other intrinsic neurons, thus collecting impulses from the periphery [6] and conducting them to the spinal cord as second-order neurons. Since their occurrence is restricted to a few millimeters proximal to the external anal sphincter, and therefore to a potentially important zone for recto-anal reflex mechanisms (see ref. 9 for review), it is possible that these cells might play a role in the defecation reflex.

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