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Shapes and projections of neurons with immunoreactivity for gamma-aminobutyric acid in the guinea-pig small intestine

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Summary. The distribution of nerve cell bodies and fibres with immunoreactivity for γ-aminobutyric acid (GABA) has been studied in the guinea-pig small intestine. Cell bodies were common in myenteric ganglia but were extremely rare in the submucosa. Reactive fibres were numerous in the tertiary component of the myenteric plexus and in the circular muscle but they were rare in both myenteric and submucous ganglia. Reactive nerve fibres were absent from the mucosa. This distribution conforms to previous descriptions. Exposure to exogenous GABA, in vitro, was used to supplement endogenous stores of GABA. The morphology of cell bodies was better defined after this treatment. Nearly all cell bodies had type-I morphology, i.e., the cells had numerous short lamellar dendrites and one axon. Most axons ran anally. Some could be traced to the tertiary component of the myenteric plexus, others to the circular muscle. Removal of the myenteric plexus from a short length of intestine caused a loss of nerve fibres from the circular muscle beneath the site of operation and a decrease in fibre density in the circular muscle that extended anally from the lesion for about 1 mm. The nerve lesions caused no significant changes in the tertiary plexus. It is concluded that GABA is contained in motor neurons supplying the longitudinal and circular muscle, and that the neurons supplying the circular muscle may be inhibitory.

Key words: γ-Aminobutyric acid – Enteric nervous system – Intestine, small – Neurotransmitters – Guinea-pig

Some intestinal neurons can be revealed by autoradiography following exposure of the intestine to radiolabelled γ-aminobutyric acid (GABA) (Jessen et al. 1979; Krantis and Kerr 1981; Saffrey et al. 1983; Krantis et al. 1986). Endogenous GABA immunoreactivity can also be seen in enteric neurons by immunohistochemical methods utilizing antibodies against GABA (Jessen et al. 1986; Saito and Tanaka 1986; Davanger et al. 1987; Hills et al. 1987). It has been assumed that the neurons revealed by autoradiography and by immunohistochemistry are the same. GABA neurons appear to be wide-spread in the enteric nervous system. Cell bodies and fibres have been found in the stomach of the rat and in the small and large intestines of rat and guinea-pig (Jessen et al. 1978; Krantis and Kerr 1981; Saito and Tanaka 1986; Hills et al. 1987); cell bodies and fibres have been observed in the chicken gizzard and quail small intestine (Saffrey et al. 1983; Baetge and Gershon 1986); nerve fibres have been reported in rat stomach (Hills et al. 1987), and cat stomach, small and large intestine (Davanger et al. 1987; Hills et al. 1987). Immunohistochemical methods are less sensitive in revealing cell bodies than nerve fibres, possibly because GABA is more actively synthesized in terminals than in cell bodies (Davanger et al. 1987; Hills et al. 1987), and in some studies cell bodies were not seen. On the other hand, although autoradiography after loading reveals cell bodies effectively, the spread of autoradiographic silver grains tends to obscure cell body shape (Krantis et al. 1986). Consequently, the shapes of GABA neurons have not been adequately described. Moreover, their projections have not been worked out, although Hills et al. (1987) have described some immunoreactive fibres that ran from myenteric ganglia into the circular muscle.

Recent studies show that the shapes, chemical distinctions and physiological properties of enteric neurons are correlated (Bornstein et al. 1984; Erde et al. 1985; Katayama et al. 1986; Iyer et al. 1988). Moreover, the projections of the neurons clearly provide clues to their functions. In the present work, we have combined exposure to exogenous GABA, which increases intracellular levels of GABA, with immunohistochemical localization so that we could reveal cell shape with fidelity. Nerve lesions were used to determine projections of the GABA neurons.

Materials and methods

The following procedure was adopted as standard to reveal GABA immunoreactivity; observations made when this procedure was varied are noted in the text. Segments of small intestine were quickly removed after guinea-pigs (150–300 g) had been killed by being stunned and having their carotid arteries severed. The segments of small intestine were placed in culture medium, consisting of Dulbecco's modified Eagle's medium with Ham's nutrient mixture (medium DME-F12, from Sigma Corp., St. Louis, Mo). The medium was kept at 37° C and buffered with medical air containing 5% CO_2. To this medium was added: nicardipine (3×10^{-5} M), to prevent muscle contraction; aminooxyacetic acid (2×10^{-5} M), to block GABA-transaminase

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activity; and β-alanine (10^{-3} M), to prevent GABA uptake into glial cells. While immersed in this medium, each segment was cut open along the mesenteric border and any contents rinsed out. It was then pinned without stretching, the muscle side uppermost, to silicone elastomer (Sylgard, Dow Corning, Michigan, USA) in a Petri dish. The preparation was maintained in this aerated medium at 37° C for a further 20 min, following which GABA (5×10^{-9} M) was added and the incubation continued for 60 min. The preparations were then re-pinned and stretched on balsa wood frames so that both surfaces were exposed. Fresh medium was used to rinse off any adherent mucus and the preparations on the frames were placed in fixative at room temperature for 3.5 h. This fixative consisted of 4% formaldehyde, 0.05% glutaraldehyde and 15% of a saturated aqueous solution of picric acid in sodium phosphate buffer, pH 7.0. The specimens were then unpinned and washed in three 10-min changes of dimethylsulphoxide, to remove the fixative, followed by three 10-min changes of phosphate-buffered saline (PBS; 0.9% NaCl in sodium phosphate buffer, pH 7.0). Tissue can be kept for up to 16 h at 4° C if convenient. The preparations were then agitated in 0.1% $NaCNBH_3$ in 0.1 M sodium phosphate buffer for 30 min, to reduce unreacted aldehyde groups, and washed in three PBS changes of 15 min each and four subsequent 1 h changes, with agitation, in PBS. Tissue was transferred to PBS containing 0.1% NaCN (for whole mounts) or 0.1% NaCN plus 30% sucrose (for sections) and stored at 4° C.

For whole mounts, the gut wall was dissected into layers: the longitudinal muscle with adherent myenteric plexus, the circular muscle, and the submucosa. Frozen sections were taken at 10–14 μm thickness. Sections or whole mounts were then incubated as follows: 30 min in PBS containing 0.1% merthiolate plus 20% normal goat serum; overnight in primary antiserum diluted in the same solution; 6 washes of 5 min each in PBS. Sites of antibody binding were located either with an avidin-biotin-HRP method, using the ABC kit from Vector Laboratories (Burlingame, California), or using a biotin-streptavidin-Texas red conjugate (1:50, also from Vector).

The primary antisera against GABA were antiserum R10A used at 1:1600 (characterized by Maley and Newton 1985), antiserum 26 used at 1:100–1:200 (characterized by Ottersen and Storm-Mathisen 1984), and antiserum AB141 (Chemicon) used at 1:100. Absorption controls were carried out according to the protocols that have been devised and validated by Ottersen et al. (1986). A conjugate of GABA and glutaraldehyde was prepared by mixing GABA (20 μmol) and glutaraldehyde (40 μmol) in 100 μl of 0.1 M sodium phosphate buffer (pH 7.4). Free aldehyde groups were then blocked by adding excess aminoethanol. Diluted antisera were mixed with the complex, to give a final concentration of amino acid of 10^{-4} M. The mixture of complex and antiserum was left to equilibrate for 20 h at 4° C. No staining of neurons was obtained with the absorbed antisera.

Operations were performed on 4 guinea-pigs to sever nerve pathways running within the small intestine. The guinea-pigs were anaesthetized with a mixture of sodium pentobarbitone (15 mg/kg) fentanyl (0.2 mg/kg) and droperidol (10 mg/kg). A segment of small intestine was exteriorized through a mid-line incision and a myectomy performed by removing the longitudinal muscle and underlying myenteric plexus from the full circumference of 3 to 6 mm of the intestine (Furness and Costa 1979). The small intestine was returned to the abdominal cavity, the abdominal wall was sutured, and the animals allowed to recover for 2 days (2 animals) or 4 days (2 animals).

Results

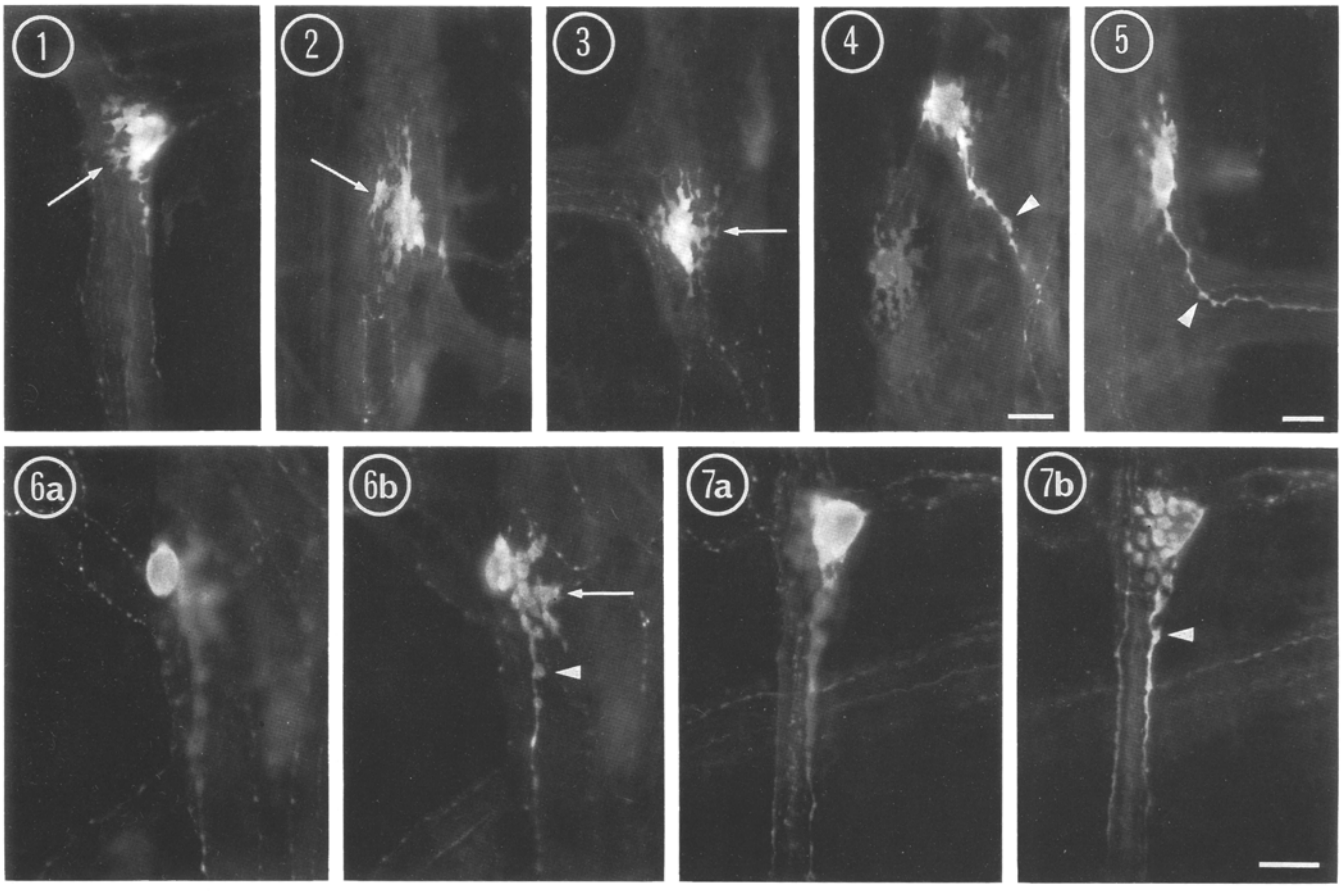
Effects of loading GABA stores

Nerve cell bodies and nerve fibres were detected immunohistochemically in the wall of the small intestine that was fixed without any pretreatment. Exposure to GABA (5×10^{-9} M) in the presence of the GABA transaminase inhibitor, aminooxyacetic acid (2×10^{-5} M), and the inhibitor of glial cell GABA uptake, β-alanine (10^{-2} or 10^{-3} M), prior to fixation, increased the intensity of the reaction but did not change the distribution of immunoreactive nerve cell bodies or fibres. Nevertheless, the morphology of each cell body was much more clearly defined after loading with GABA. In particular, lamellar dendrites that could be seen only faintly in preparations without loading were strongly reactive in the loaded preparations. Omission of β-alanine resulted in no or only weak reactivity in glial cells of the myenteric plexus, suggesting that these glial elements have little facility to take up and retain exogenous GABA under the conditions of the present experiments. In contrast, there was significant reactivity in the glial cells of submucous ganglia and glial cells in the nerve bundles following submucous arterioles in preparations exposed to GABA without β-alanine. The same distributions of nerve cells and fibres were observed with each of the three antisera used.

Morphology and distribution of nerve cell bodies

Immunoreactive nerve cells were found in almost all myenteric ganglia. By shape, they could be ascribed to the Dogiel type-I classification (Dogiel 1899) when viewed in whole mounts of small intestine. The cell bodies had numerous short, broad processes that were generally flattened in the plane of the plexus and one long, finer process (Figs. 1–7). These processes correspond to the lamellar dendrites and axons, respectively, that are features of neurons with type-I morphology (Dogiel 1899). The lamellar dendrites were sometimes in the same plane as the part of the cell body containing the nucleus, but were also found in a plane either above or below the nucleus when viewed in whole-mount preparations (Figs. 6, 7). In a few instances there were lamellar dendrites both above and below the plane of the cell body. Cell bodies at the lateral edges of ganglia often had a majority of prominent dendritic processes that protruded into the body of the ganglion (Fig. 7), or the cell bodies were elongated as if they were compressed against the ganglion surface. The initial parts of the axons (up to about 100 μm from the cell body) often bore short protuberances (axonal spines; Figs. 5–7). Those few cells that we were unable to classify were either obscured or were flattened at the sides of ganglia and their processes were not easy to distinguish. No immunoreactive nerve cells had Dogiel type-II morphology, i.e., there were no neurons with smooth cell bodies and multiple long processes.

The majority of cell bodies were in the prominent ganglia of the plexus, but a few were found at intersections of primary and secondary strands of the plexus or lying



Figs. 1–7. Shapes of neurons displaying GABA immunoreactivity in the myenteric plexus of the guinea-pig small intestine (whole-mount preparations). These neurons have type-I morphology in the classification of Dogiel (1899). The lamellar dendrites that are typical of these neurons are indicated by *arrows* in **Figs. 1–3** and **6b**. *Arrowheads* point to axonal spines in **Figs. 4, 5, 6b** and **7b**. The pairs of micrographs, **Figs. 6** and **7**, each show a single cell at two levels of focus. For these two cells the part of the cell body containing the nucleus has a smooth outline and the dendrites are in a different plane of focus. Few varicose fibres immunoreactive for GABA occur in myenteric ganglia. Calibration: 20 μm (calibration on Fig. 4 refers to Figs. 1–4; calibration on Fig. 7b refers to Figs. 6 and 7)

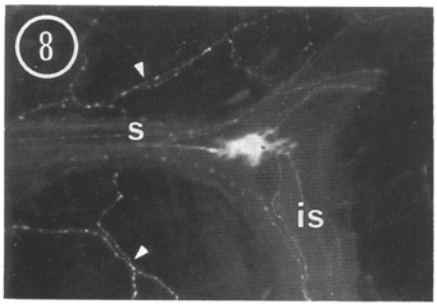
along secondary strands (Figs. 4, 5, 8). Some cell bodies at intersections or in the secondary strands had an axon emerging from one pole and the majority of their dendritic processes emerging from the opposite pole. In two preparations in which counts were made, 169 cell bodies were in ganglia and 14 were at intersections or within secondary strands. Nerve cell bodies were nearly always at the surfaces of ganglia. This could be seen in whole mounts by focussing through the ganglia (Fig. 9) and in sections (Figs. 10–12). More cells were at the circular muscle surface than at the longitudinal muscle surface. Counts in two preparations gave 85 cell bodies at the circular muscle surface and 32 at the longitudinal muscle surface. In 62 ganglia from 4 preparations there were 315 reactive cell bodies, an average of 5.1 cell bodies per ganglion, with a standard deviation of 3.6.

Cell bodies were very rarely found in submucous ganglia in either GABA loaded preparations or in preparations not exposed to GABA. Thirteen reactive cell bodies were found in 510 ganglia from 6 preparations.

Nerve fibre distribution

Reactive nerve fibres were very sparse within myenteric ganglia (Figs. 14–16). The majority of fibres ran along or through the ganglia without seeming to form specific associations, such as baskets of varicosities, in relation to nerve cell bodies. Most of the fibres were at or near the surfaces of ganglia (e.g., Fig. 16). These fibres were usually varicose. They could frequently be followed from one connecting nerve fibre strand, across a ganglion, and into another strand. A small proportion of fibres in internodal strands (Fig. 17) and in the secondary strands of the plexus (Fig. 18) were immunoreactive.

Numerous varicose fibres followed the small nerve fibre bundles of the tertiary component of the myenteric plexus, which lies in the spaces between the ganglia and principal connecting strands of the plexus (Fig. 19). Reactive fibres could be traced to the tertiary plexus from secondary strands (Fig. 20) and from the ganglia and internodal strands of the primary plexus. The innervation of the circular muscle by GABA-immunoreactive fibres was dense (Fig. 21). Numerous fibres were in the deep muscular plexus, where they appeared to be in all the nerve bundles, and fibres running parallel to the muscle were found throughout the thickness of the circular muscle coat.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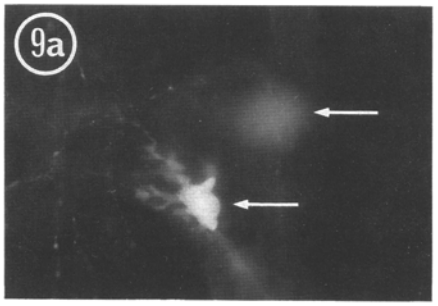
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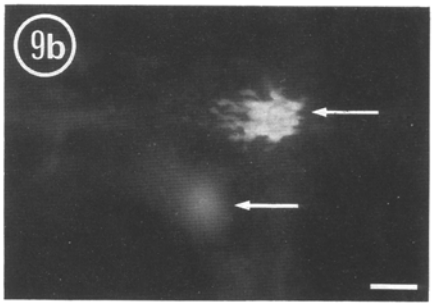
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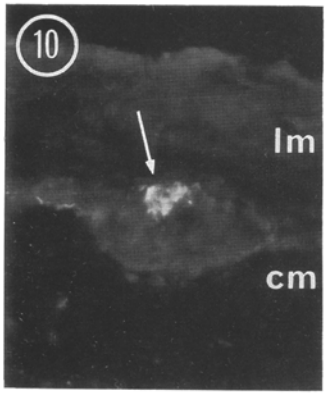
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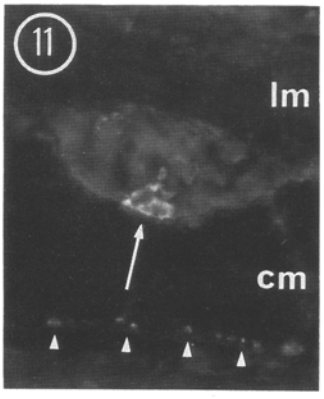
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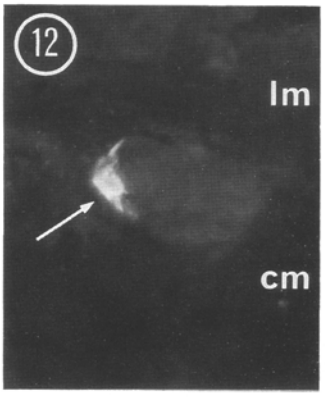
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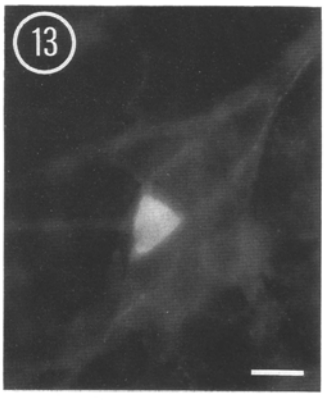
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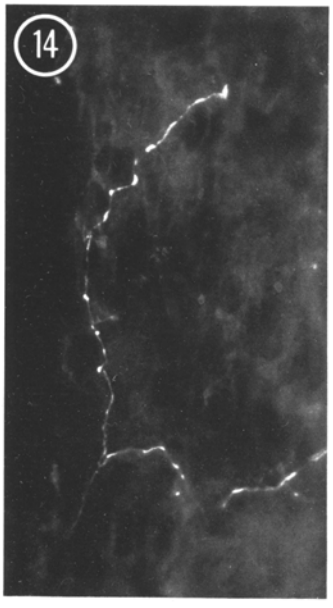
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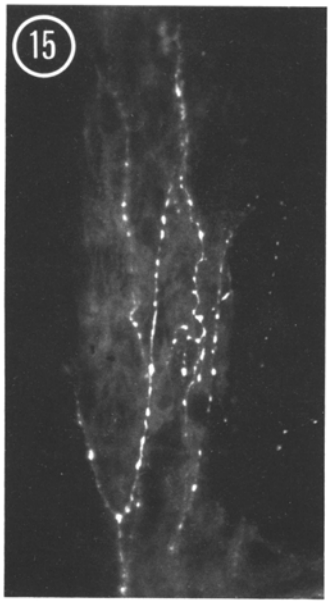
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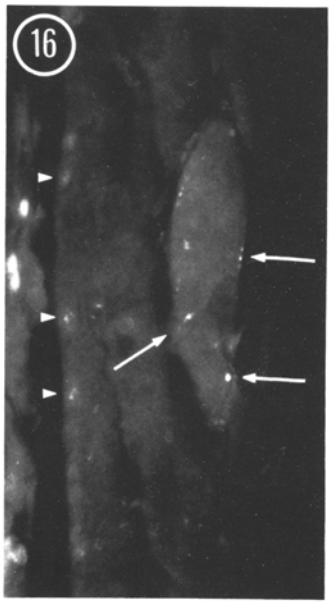
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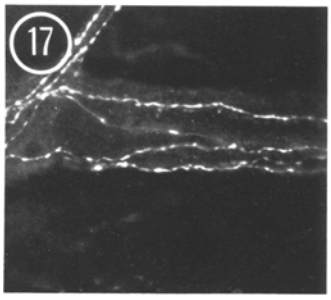
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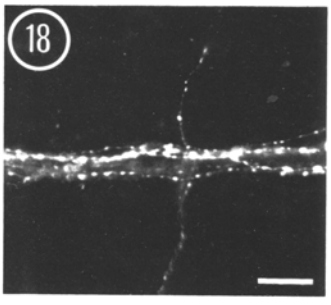
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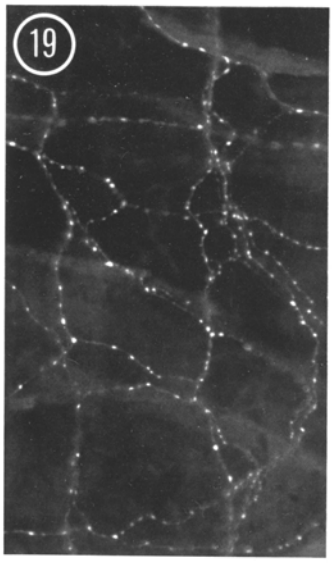
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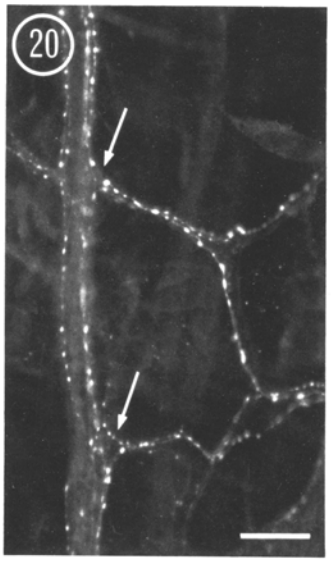
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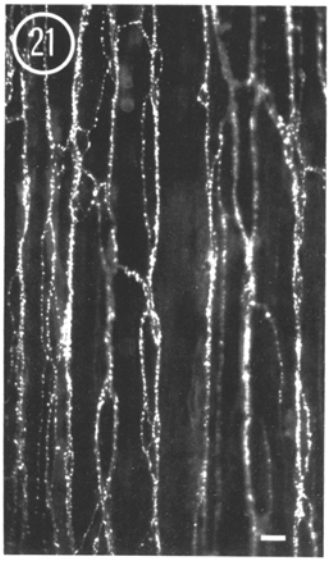
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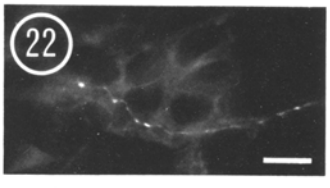
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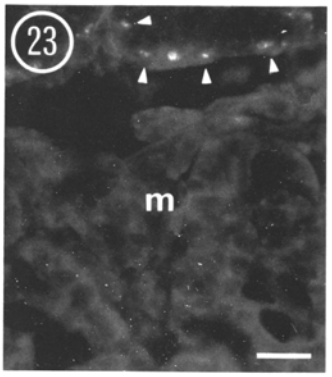
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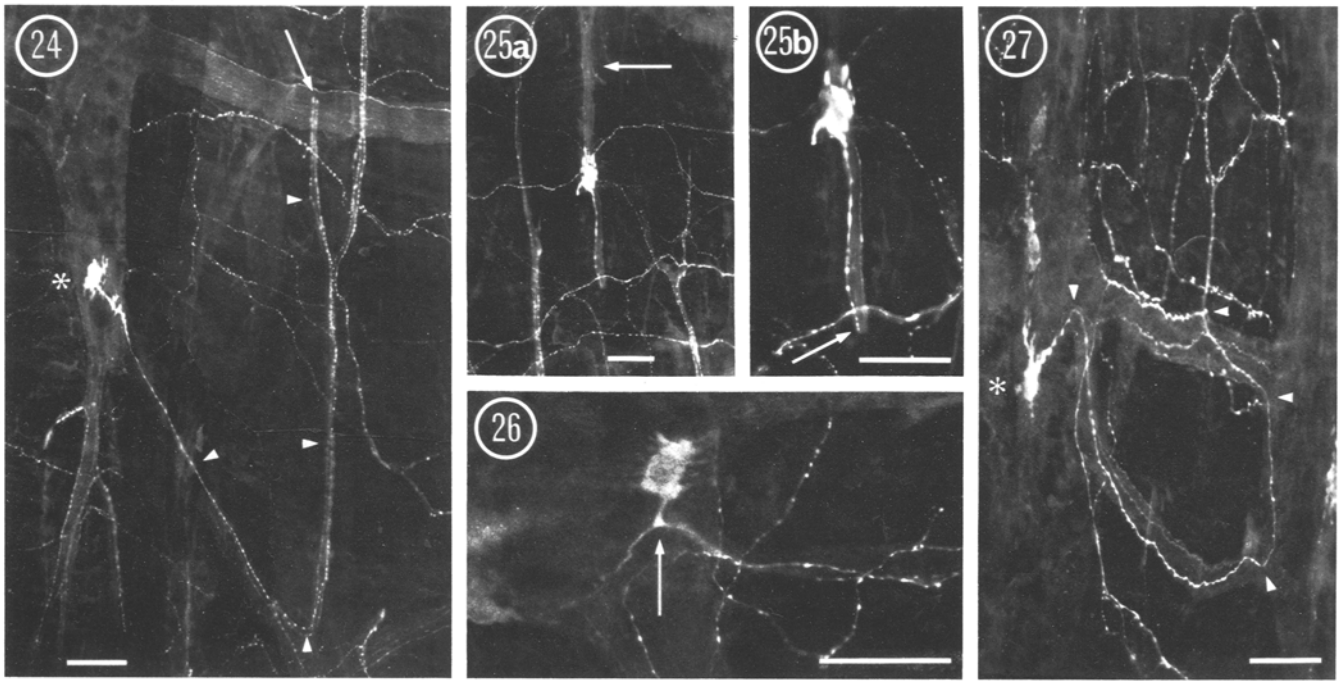


Figs. 24–27. Axons of GABA-immunoreactive neurons in the myenteric plexus followed in whole mounts. **Fig. 24.** Nerve cell body in a myenteric ganglion (*asterisk*), whose axon can be followed anally (*arrowheads*) into a secondary strand of the myenteric plexus that is broken off where it enters the circular muscle coat (*arrow*). **Fig. 25** Nerve cell body that lies within a secondary strand of the myenteric plexus (*arrow*, **Fig. 25a**). The axon of this nerve cell can be followed circumferentially to the broken end of the secondary strand where it enters the circular muscle (*arrow*, **Fig. 25b**). The nerve cell body in **Fig. 26** has an axon that bifurcates at the *arrow*. The process that runs to the right entered the tertiary component of the myenteric plexus. The nerve cell indicated by the *asterisk* in **Fig. 27** has a single axon (*arrowhead*) that can be followed into the tertiary plexus just anal to the ganglion in which the nerve cell lies. Calibrations: 50 μm

In the majority of ganglia of the submucous plexus no reactive fibres could be found. However, there were a few non-varicose fibres that ran through the plexus and across some ganglia (Fig. 22), and rare varicose fibres were encountered. No nerve fibres could be found in the mucosa (Fig. 23).

Projections from myenteric nerve cells

Because the density of nerve fibres in the myenteric ganglia was low, it was possible to trace the long (axonal) processes of most individual cell bodies (Figs. 24–28). With very few exceptions, each cell had only one axon; it sometimes

Figs. 8–13. Positions of GABA-immunoreactive nerve cells in myenteric ganglia. **Fig. 8.** Cell body at the intersection of an internodal strand (*is*), and a fibre bundle of the secondary component of the plexus (*s*). *Arrowheads* indicate fibres of the tertiary plexus. **Fig. 9.** Myenteric ganglion from a whole-mount preparation at two different levels of focus. Corresponding *arrows* point to the same cell bodies in and out of focus. The cell in focus in **Fig. 9a** is at the longitudinal muscle surface, that in **9b** is at the circular muscle surface. **Figs. 10–12.** Sections showing that reactive cell bodies (*arrows*) are almost always at the surfaces of the ganglia; *lm* longitudinal muscle; *cm* circular muscle; *arrowheads* fibres at the deep muscular plexus. The ganglia appear lighter than the surrounding muscle. **Fig. 13.** Reactive nerve cell body, but no reactive fibres, in a submucous ganglion. Calibrations: 20 μm (calibration on Fig. 9b refers to Figs. 8 and 9; calibration on Fig. 13 refers to Figs. 10–13)

Figs. 14–23. Distribution of nerve fibres with immunoreactivity for GABA. **Figs. 14, 15.** Myenteric ganglia in whole mounts. Usually only single fibres, which course across or through the ganglia, are found (**Fig. 14**). The maximum densities of fibres are similar to that shown in **Fig. 15**. In a section through a ganglion, only a small number of reactive fibres is found (**Fig. 16**). *Arrows* represent reactive fibres in the ganglion; *arrowheads* show fibres in the deep muscular plexus. **Figs. 17, 18.** Reactive fibres in an internodal strand (**17**) and in a secondary strand (**18**) of the myenteric plexus. **Fig. 19.** The tertiary component of the myenteric plexus. **Fig. 20.** Reactive fibres run from secondary strands into the tertiary plexus (*arrows* indicate junctions between the two components). **Fig. 21.** Nerve fibres in the circular muscle shown in a whole-mount preparation. The fibres run parallel to the long axes of the muscle fibres. **Fig. 22.** A single reactive nerve fibre coursing through a submucous ganglion. **Fig. 23.** Section through the inner part of the circular muscle and the mucosa, showing fibres in the deep muscular plexus (*arrowheads*) and the lack of reactive fibres in the underlying mucosa (*m*). Calibrations: 20 μm (calibration on Fig. 18 refers to Figs. 14–19)

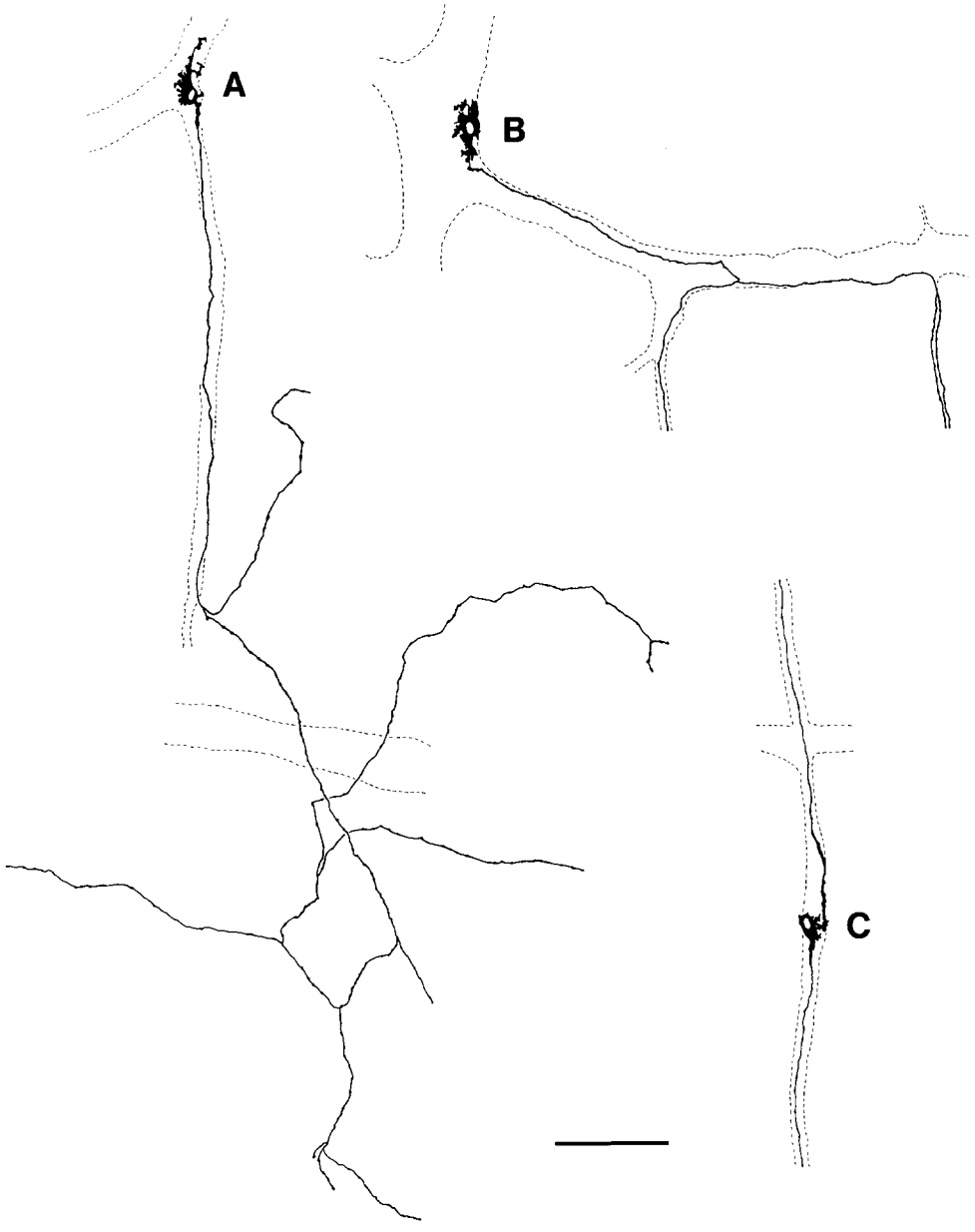
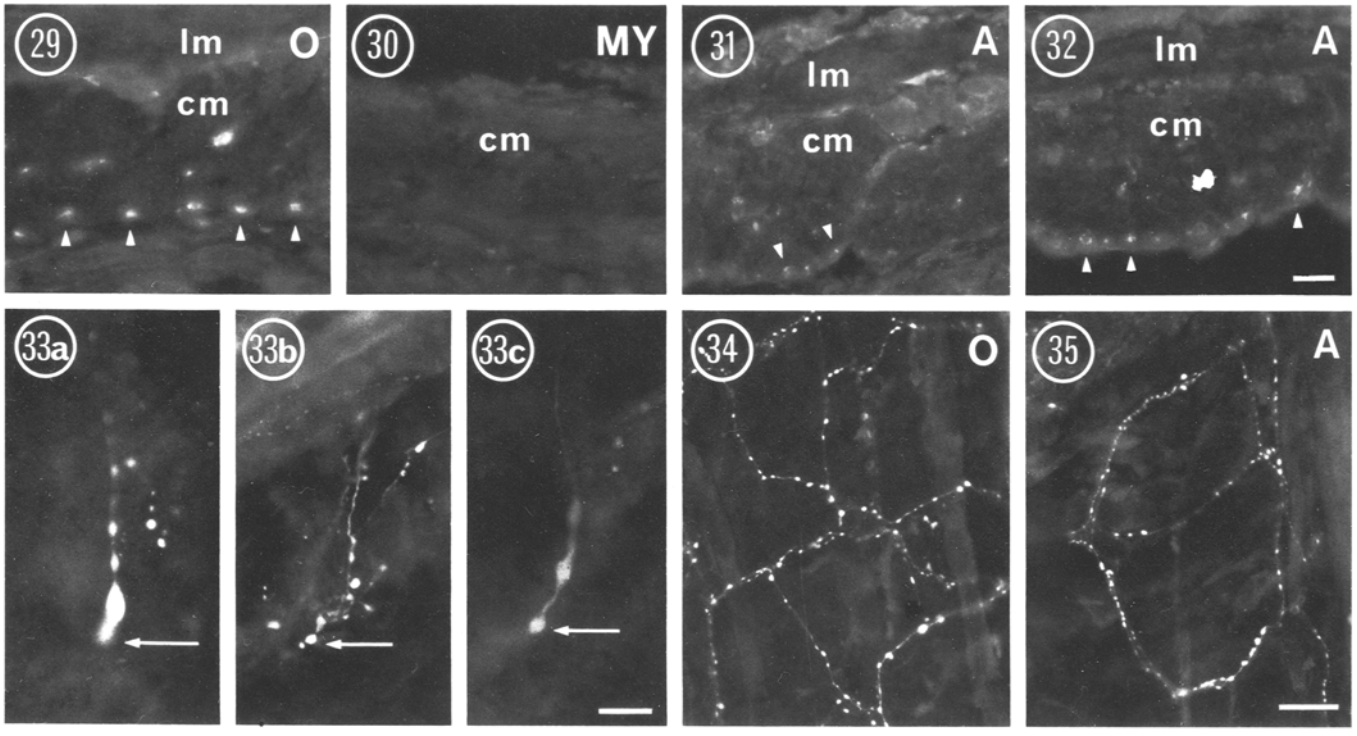


Fig. 28. Camera lucida drawings of three GABA-immunoreactive neurons in the myenteric plexus. These drawings were made from whole mounts in which the immunoreactivity was localized by an avidin-biotin-HRP method. Oral is to the left, anal to the right. The *dotted lines* in these drawings indicate the outlines of ganglia and nerve strands. Cell *A* lay at the end of a myenteric ganglion and gave rise to a single long process that followed a secondary strand before entering the tertiary component of the myenteric plexus. It gave rise to a loose network of branches in the plexus. Cell *B* sent its axon anally and, after branching, it followed secondary strands that were broken off where they entered the circular muscle. Cell *C* lay in a secondary strand and was one of the rare bipolar cells that was found. Its axons ran to the broken ends of secondary strands entering the circular muscle. Calibration: 100 μm

branched within the ganglion of its parent cell body (e.g., Fig. 26) but it usually ran into a secondary strand or into the tertiary component of the myenteric plexus before branching (Figs. 27, 28). Beyond the initial part bearing axonal spines some axons were smooth and others were varicose. All axons that could be followed left the ganglion of their cell body and nearly all travelled anally, sometimes after initially taking a circumferential course. A small proportion of axons ran orally for a short distance and then proceeded in an anal direction. The axons could be traced either to the secondary strands of the plexus or to the tertiary plexus. Secondary strands are known to carry fibres to the circular muscle (Wilson et al. 1987), although they also contain other fibres. Fibres that entered the secondary strands either branched from these to enter the tertiary plexus (Fig. 28A) or were broken off where these strands had entered the circular muscle (Figs. 25, 28B, C). It was common to encounter bifurcations of the fibres in the secondary strands or in the tertiary plexus (Figs. 27, 28). Most fibres were varicose once they entered the secondary or tertiary components of the myenteric plexus.

Following myectomy, in which a collar of longitudinal muscle and myenteric plexus was removed from the full circumference of the intestine, there was a substantial loss of reactive fibres from the circular muscle beneath the myectomy and for a short distance anal to the operation (Figs. 29–32). No change in innervation was detected oral to the site of operation. Within the area of the myectomy, beneath its oral end, a few fibres were found in the circular muscle layer, extending for about 100–150 μm from the margin of the intact region. The loss of fibres from the circular muscle could be detected for 600–1000 μm anal to the operation. There were usually very few fibres found in the first anal 600 μm, and the density of innervation was quickly restored over the next 200 to 400 μm. In whole-mount preparations, accumulations of immunoreactivity were found in nerve fibres of severed nerve strands on the oral side (Fig. 33), but not on the anal side. No change



Figs. 29–35. The effects of myectomy lesions on GABA-immunoreactive nerve fibres. **Figs. 29–32.** Sections showing reactive fibres in the muscle 4 days after myectomy. **Fig. 29** is from 500 μm oral (*O*) to the operation; **Fig. 30** is from beneath the area of myectomy (*MY*); **Fig. 31** is 50 μm anal (*A*); and **Fig. 32** is 800 μm anal (*A*) to the operation. *Arrowheads* point to fibres of the deep muscular plexus; *lm* longitudinal muscle; *cm* circular muscle. **Fig. 33a–c** are whole mounts showing accumulations of immunoreactivity in severed nerve strands of the oral margins of myectomies two days after the operations; *arrows* show where the axons have been severed. **Figs. 34, 35** are of the tertiary plexus, 100 μm oral (34) and 50 μm anal (35) to a myectomy performed 2 days before. Calibrations: 20 μm (calibration on Fig. 32 refers to Figs. 29–32; calibration on Fig. 33c refers to Fig. 33a, b, c; calibration on Fig. 35 refers to Figs. 34 and 35)

in the fibre distribution in the tertiary plexus was found, even within 50 to 100 μm of the operated region (Figs. 34, 35).

Discussion

GABA immunoreactivity occurred in nerve cell bodies in the myenteric ganglia, and the most numerous reactive nerve fibres were in nerve strands and the tertiary component of the myenteric plexus and in the circular muscle coat of the guinea-pig small intestine. A similar distribution of nerve cells and fibres has previously been observed by the autoradiographic localization of exogenous GABA and by the immunohistochemical localization of endogenous stores (Jessen et al. 1979, 1986; Krantis and Kerr 1981; Krantis et al. 1986; Saito and Tanaka 1986; Hills et al. 1987). The present work suggests that the neurons that take up and retain exogenous GABA are the same neurons that contain endogenous GABA.

From direct tracing of the processes of GABA neurons, and from observations of the effects of lesions, it appears that there are two groups of GABA neurons: one group supplying the longitudinal muscle via the tertiary plexus, and the other supplying the circular muscle. Uniaxonal neurons, such as these, that project to the muscle are likely to be motor neurons. The cell bodies have Dogiel type-I morphology, a shape that is indicative of their being, by electrophysiological classification, S neurons (Bornstein et al. 1984; Katayama et al. 1986). Considerable circumstantial evidence indicates that motor neurons to the muscle are S neurons (Furness et al. 1988).

The projections of the neurons innervating the circular muscle suggest that the GABA-immunoreactive neurons may be inhibitory motor neurons. Electrophysiological studies of transmission from motor neurons to the circular muscle indicate that it receives both excitatory and inhibitory inputs (Taylor and Bywater 1986). The excitatory neurons run orally within the intestine (Smith et al. 1988), while the inhibitory neurons run anally (Bornstein et al. 1986). Two distinct classes of inhibitory neurons were identified: the majority projects anally for 1 to 1.5 mm or less, while others project anally for up to 30 mm (Bornstein et al. 1986). Thus, the projections of the GABA neurons to the circular muscle are very similar to those of the shorter type of inhibitory motor neurons.

Although the axons of the GABA neurons could be traced to the circular muscle where they join circumferentially running nerve fibre bundles, the distances that individual nerve fibres run within these bundles could not be determined histochemically. However, the decline in amplitude of inhibitory junction potentials recorded in the muscle with increasing distance from stimuli applied to circumferentially directed pathways suggest that the fibres run for up to about 11 mm and that most have lengths of 6 to 8 mm (Bornstein et al. 1986). Excitatory motor neurons project

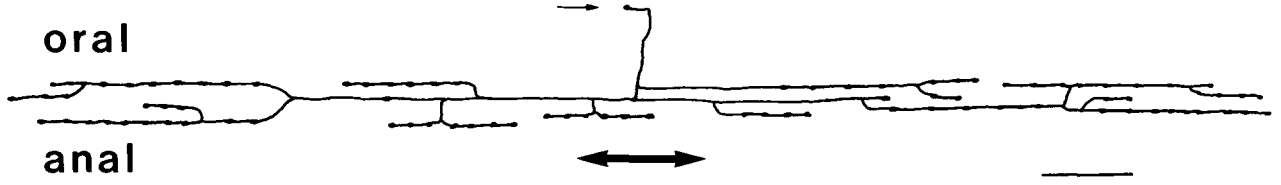


Fig. 36. Reconstruction of the projection to the circular muscle of a typical GABA-immunoreactive neuron, as would be seen by looking down through the surface of a segment of intestine that was opened and laid out flat. The cell body (*arrow*) is in the myenteric plexus and gives rise to an axon that runs anally for about 0.5 mm before providing a series of circumferentially directed processes in the circular muscle. The circumferential direction is marked by the *double-headed arrow*. Calibration: 0.5 mm

circumferentially for a similar distance (Smith et al. 1988). The structural information from the present paper, along with the physiological determination of circumferential projections, leads to the conclusion that the GABA-reactive neurons projecting to the circular muscle have the shape shown in Fig. 36. Our observations suggest that separate neurons give rise to the varicose fibres with GABA immunoreactivity in the tertiary component of the myenteric plexus, which is believed to provide the motor innervation of the longitudinal muscle.

The role that GABA plays in transmission to the smooth muscle is not known. However, it is unlikely that this compound directly influences the excitability of the muscle. The effects of GABA have been examined in several regions of the intestine and in each the muscle has appeared to be quite insensitive (Holbinger 1958; Krantis et al. 1980; Krantis and Kerr 1981; Kleinrock and Kilbinger 1983). GABA does, however, modify transmission from enteric neurons (Kleinrok 1982; Ong and Kerr 1983, 1984; Ohkawa 1987), so that its effects might be on the terminals of other neurons innervating the muscle, as Jessen et al. (1983, 1987) have suggested previously. It is also possible that GABA released from terminals within the muscle may require the presence or co-release of another transmitter to exert an effect on the muscle.

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