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MECHANORECEPTOR PATHWAYS FROM THE DISTAL COLON TO THE AUTONOMIC NERVOUS SYSTEM IN THE GUINEA-PIG

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SUMMARY

1. Electrophysiological and histological techniques were used to trace sensory pathways for stretch mechanoreceptor fibres from the distal colon to dorsal root ganglia.

2. Extracellular and intracellular recording techniques revealed sensory pathways for mechanoreceptors to the prevertebral sympathetic ganglia but no further centrally.

3. Histological studies involving the retrograde transport of horseradish peroxidase revealed sensory pathways from the distal colon to the spinal cord, mainly to the level of the second lumbar vertebra. Few (< 2000) fibres were involved; their perikarya were small (*ca.* 25 μm).

4. Sensory perikarya in spinal ganglia in the guinea-pig could be categorized into two populations, F and H cells, after a previously defined nomenclature for murine spinal ganglion cells.

5. F and H cells were distinguished initially by their times to decay by 50% of the action potential. H cells took three times as long to repolarize.

6. F and H cells were distinguished further by their electrical properties including membrane potential, input resistance and amplitude and duration of the after-potential following the action potential.

7. Both F and H cells showed unusual time-dependent rectification following either depolarizing or hyperpolarizing current pulses. Threshold currents to show rectification were different for F and H cells.

8. When taken in conjunction with conduction velocities, the electrophysiological evidence may assist in identifying sensory neurones. For example, H cells appeared to have slow conducting (C fibre) axons.

9. From the lack of electrophysiological evidence and limited histological support for major central sensory pathways, it is concluded that stretch mechanoreceptor information from the colon of the guinea-pig is referred mainly to the prevertebral ganglia with minimal involvement of the spinal cord.

INTRODUCTION

Decentralization of the prevertebral ganglia fails to alter to any great extent the degree of sympathetic tone to the colonic musculature (Garry, 1933; Lawson & Holt, 1938; Kuntz & Van Buskirk, 1941; deGroat & Krier, 1979). It has been suggested that this continued sympathetic tone probably involves a peripheral reflex where sensory information is relayed directly from the colon to sympathetic neurones in prevertebral ganglia (Chang & Hsu, 1942; Kuntz & Saccamano, 1944; Crowcroft, Holman & Szurszewski, 1971; deGroat & Krier, 1979). One type of sensory neurone, the stretch mechanoreceptor, is known to make cholinergic synapse with principal sympathetic ganglion cells of the prevertebral ganglia (Szurszewski & Weems, 1976; Kreulen & Szurszewski, 1979). These mechanoreceptors can be activated with radial distension of the colon (Kuntz & Van Buskirk, 1941). It has been suggested that their perikarya may lie in the colon wall (Ross, 1954; Ungávy & Léränth, 1970; Dalsgaard & Elfvin, 1982) and that C fibres project centripetally from these cell bodies directly to the prevertebral ganglia (Ungávy & Léränth, 1970). Furthermore, peripheral C fibres have been shown to make axodendritic cholinergic synapses with sympathetic neurones (Archakova, Bulygin & Netukova, 1982). Taken together, these observations suggest the presence of a peripheral sensory limb in an autonomic reflex arc which is totally unconnected with the central nervous system.

This study was undertaken to determine whether stretch mechanoreceptor information from the colon of the guinea-pig is relayed exclusively to prevertebral ganglia by such sensory neurones or if the spinal cord might also share in their sensory information. Our experiments involved the use of intracellular as well as extracellular recording techniques to trace sensory nerve tracts. Intracellular recordings were made at a prevertebral ganglion (the inferior mesenteric ganglion; i.m.g.) and at dorsal root ganglia (d.r.g.), at the levels of thoracic (T_{13}) and lumbar (L_1–L_4) spinal segments. Extracellular recordings of multiunit axonal discharge were made on peripheral and central nerve pathways between the spinal cord and distal colon. To complement these electrophysiological studies, horseradish peroxidase was applied to peripheral nerves and used to detect sensory pathways leading back to spinal ganglia. The data obtained strongly suggest that there is an absence or extreme paucity of stretch mechanoreceptor cells in dorsal root ganglia and that sensory information carried by colonic mechanoreceptors is referred mainly to the prevertebral ganglia with minimal involvement of the spinal cord.

METHODS

Male guinea-pigs (500–600 g body weight) were anaesthetized with sodium pentobarbitone (90 mg/kg) given intraperitoneally. The thoracic cavity was opened, descending aorta cannulated and the animal perfused (40 ml/min) with chilled (4 °C) physiological saline; the inferior vena cava was cut to facilitate drainage of the vascular bed. A laminectomy was carried out from the twelfth thoracic vertebra (T_{12}) to the fifth lumbar vertebra (L_5). Thereafter, the abdominal muscles were removed, as well as the kidneys and adrenal glands, and the distal colon cut at the splenic flexure and pelvic brim, down to the retroperitoneal wall. Finally, the spinal column was severed above T_{12} and below L_5 and isolated tissues between those points transferred to a large dissecting dish containing chilled physiological saline. The skeletal muscles forming the retroperitoneal wall were stripped away revealing the left sympathetic paravertebral chain and spinal nerves. Then the

transverse processes on the vertebrae were removed and the dura mater cut to allow access to the left dorsal and ventral roots and dorsal root ganglia. The preparation is shown diagrammatically in Fig. 1.

Preparations were placed in a twin-chambered bath, the colon in one chamber with the mesentery draped over a connecting wall and the spinal column placed in a second, front chamber. The colon was tied off at its proximal end and cannulated at its distal end to distend the gut with air and activate intrinsic stretch mechanoreceptors. The colon and mesentery draping the connecting wall

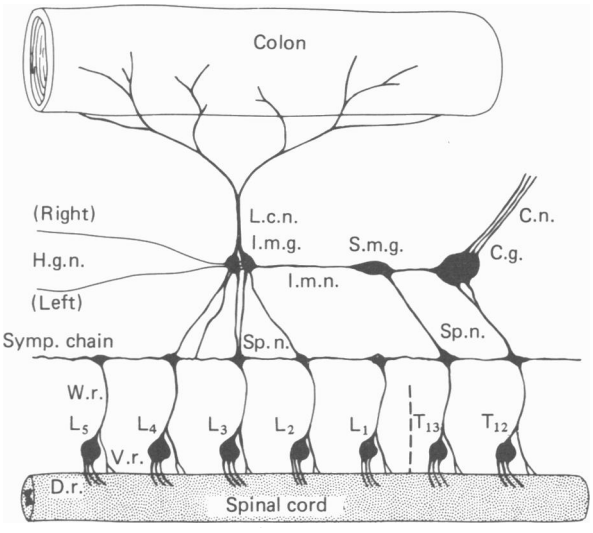

Fig. 1. Diagram of experimental model showing central and peripheral nerve pathways to prevertebral ganglia of the guinea-pig. Central nerve pathways included dorsal (d.r.) and ventral (v.r.) roots, from the levels of thoracic (T_{12} and T_{13}) and lumbar (L_1–L_5) vertebral segments, and sympathetic white rami (w.r.) connecting spinal roots to the left sympathetic chain. Thoracic and lumbar splanchnic nerves (sp.n.) connect these central nerves to prevertebral ganglia. The prevertebral ganglia are the inferior mesenteric ganglion (i.m.g.); superior mesenteric ganglion (s.m.g.); coeliac ganglia (c.g.). Peripheral nerves in the mesentery include the left and right hypogastric nerves (h.g.n.), intermesenteric nerve (i.m.n.), coeliac nerve (c.n.) and lumbar colonic nerve (l.c.n.). The same abbreviations are used in subsequent Figures.

were covered with moist tissue-paper to prevent desiccation. Both chambers were perfused separately with a modified Krebs physiological solution containing (mM): Na^+, 137·4; K^+, 5·9; Ca^{2+}, 2·5; Mg^{2+}, 1·2; Cl^-, 134; HCO_3^-, 15·5; $H_2PO_4^-$, 1·2; glucose, 11·5; equilibrated with 97 % CO_2 and 3 % CO_2 and maintained at 35–37 °C.

Bipolar silver/silver chloride stimulating electrodes were placed on central and peripheral nerve pathways. Central nerves included spinal roots, spinal sympathetic rami and lumbar-splanchnic nerves. Peripheral nerves included hypogastric and lumbar-colonic nerves, the intermesenteric nerve as well as the coeliac (superior and inferior) nerve bundles; stimulus isolation units were used throughout. Pulse duration was 10–500 μs and supramaximal voltage was used.

Intracellular potentials were recorded using glass micro-electrodes filled with 3 M-KCl (30–80 MΩ tip resistance). The micro-electrode was connected to an electrometer with an active bridge circuit to allow current to be passed through the recording electrode. Recorded signals were either photographed or stored on tape for further analysis. Impalement of a neurone in the inferior mesenteric ganglion and in a spinal ganglion was deemed satisfactory if the recorded potential showed an abrupt negative deflexion in excess of −40 mV which was either maintained or increased in magnitude and if the neurone supported an action potential overshooting zero potential.

Multiunit discharge was recorded from nerve bundles using extracellular recording techniques. Bipolar silver/silver chloride recording electrodes were connected to an a.c. pre-amplifier. Signal passband was 1 Hz–3 kHz half-amplitude and signal amplification was 50000. Recorded signals were handled as described above. For extracellular recording, the front chamber containing the spinal column was flooded with warm (37 °C) liquid paraffin. To interrupt transmission at any nicotinic synapse the paraffin was replaced by physiological saline containing hexamethonium bromide (Sigma) at a concentration of 5×10^{-4} M for 1 h; then the chamber was flooded again with liquid paraffin for further experimentation.

Electrophysiological data were expressed as a mean ± one standard error of the mean (S.E. of mean) for those observations. Sets of data were compared using a two-tailed Student's t test. Differences between unpaired sets of data were considered significant if P values were less than or equal to 0·05.

Neuroanatomy

Afferent pathways from the lumbar-colonic nerves to dorsal root ganglia were mapped using horseradish peroxidase tracing techniques. Animals were anaesthetized with ketamine hydrochloride (44 mg/kg body weight) administered intraperitoneally. A mid-line incision was made through the abdominal wall and the lumbar-colonic nerves were located and desheathed over a short (2–5 mm) length just peripheral to the i.m.g. Horseradish peroxidase (HRP; Sigma Type VI) recrystallized from a 0·9% saline solution was applied to the nerve bundles. Nerves and crystals were crushed with fine forceps and HRP was re-applied several times to the same nerve bundles. Animals were killed 60 h later and perfused via the descending aorta with 2% glutaraldehyde + 2% paraformaldehyde in 0·1 M-phosphate buffer. Left and right dorsal root ganglia from T_{12} to L_5 were excised and sections (50 μm thickness) developed to test for retrograde axonal transport of HRP by sensory afferent fibres to cell somata. The HRP-reaction product was developed using the diaminobenzidine (DAB) method as outlined by Adams (1977). The diameters of dorsal root ganglion cells in sections prepared for HRP-staining were measured using a particle size analyser. Both unstained and stained cells were measured. The criteria for morphometric analysis by Offord, Ohta, Eonning & Dyck (1974) were adopted.

RESULTS

Intracellular recording at the i.m.g.

To be certain all peripheral and central nerve pathways were intact physiologically, intracellular recordings were made from sympathetic neurones in the i.m.g. and synaptic input to these cells was observed following electrical stimulation of various nerve pathways as well as during on-going synaptic input from colonic mechanoreceptors. In all preparations, a single volley applied in turn to the lumbar-colonic nerves, left hypogastric nerve, intermesenteric nerve and inferior and superior coeliac nerves consistently evoked excitatory post-synaptic potentials (e.p.s.p.s). In general, following stimulation of any of these nerves, e.p.s.p.s summated spatiotemporally to reach threshold for firing to elicit an action potential which overshot zero potential. Each peripheral nerve pathway contained numerous fibres which made synapses with the same sympathetic neurone. In all preparations the functional integrity of mechanoreceptors in the distal colon and their input to the i.m.g. were confirmed by monitoring on-going mechanoreceptor synaptic input to neurones in the i.m.g. during distension of the colon as described previously (Crowcroft, *et al.* 1971; Szurszewski & Weems, 1976). Thus, the peripheral nerve pathways in these *in vitro* preparations were functionally intact.

In all preparations, single volleys to preganglionic lumbar-splanchnic nerves (sp.n.) evoked e.p.s.p.s which summated spatiotemporally to reach a threshold for firing to elicit one or more action potentials. An example is shown in Fig. 2. In this

experiment, the preganglionic nerve supply arose from the second, third and fourth lumbar cord segments because stimulation of L_2 v.r. and L_3 v.r. and L_4 v.r. all evoked synaptic responses (Fig. 2). Similar results were obtained in nine other experiments. In all of these ten experiments, there were no synaptic responses when L_5 v.r. was stimulated. These results suggest that sympathetic neurones in the i.m.g. received a multiple innervation from preganglionic fibres not only from one spinal segment

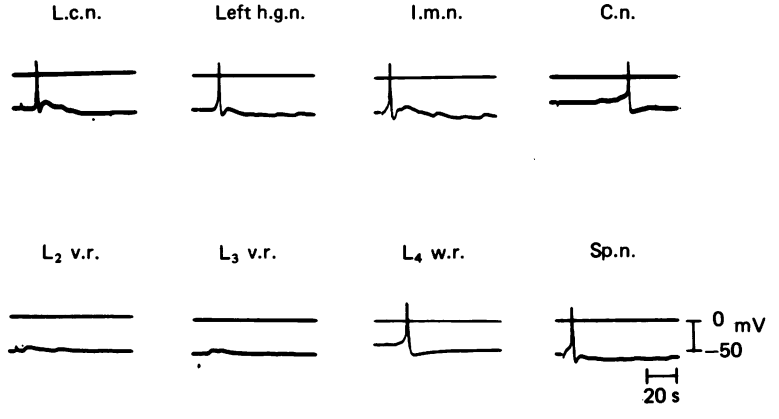

Fig. 2. Response of neurone in i.m.g. to supramaximal stimulation of peripheral (upper row) and central (lower row) nerve trunks. Spinal levels of central nerves (ventral roots and sympathetic rami) are indicated. Synaptic responses (e.p.s.p.s) summated in some cases to elicit an action potential which overshot zero potential (upper line in each record).

but over a contiguous subset of spinal segments. Similar observations have been made for sympathetic neurones in the i.m.g. of cat (Krier, Schmalz & Szurszewski, 1982). Specific segmental innervation has also been observed for superior cervical ganglion cells in the guinea-pig (Njå & Purves, 1977). Thus, by monitoring central input to ganglion cells in the present experiments the integrity of central nerve pathways was confirmed, at least from lumbar spinal roots to the i.m.g. Since these efferent nerve pathways form a common tract with visceral afferent fibres, afferent pathways were taken to be also intact. The integrity of efferent fibres allayed suspicion of damage to afferent nerve pathways during dissection.

With fifty-one i.m.g. cells in fifteen preparations, single shocks were applied to dorsal root filaments at the levels of T_{13} and L_1–L_4. Single volleys failed consistently to elicit fast cholinergic excitation (e.p.s.p.s) from all sympathetic cells tested. This observation suggests that sensory nerve fibres in dorsal root filaments do not make *en passant* cholinergic synapses with neurones in the i.m.g.

Synaptic transmission in the i.m.g. was interrupted following exposure to hexamethionium (5×10^{-4} M). Thereafter, stimulation of peripheral and central nerves failed to elicit excitatory responses from post-ganglionic neurones. However, neuronal excitability was not impaired by the nicotinic-receptor blocking agent since depolarizing current (0·1–1·0 nA) injected via the recording micro-electrode caused neurones to fire an action potential. In later experiments, hexamethonium (5×10^{-4} M) was used during extracellular recording experiments to abolish ganglionic transmission without fear of altering membrane excitability, particularly of sensory neurones.

Intracellular recording at spinal ganglia

Intracellular recordings were obtained from 176 neurones located in left d.r.g. at the levels of spinal segments T_{13} and L_1–L_4. Eighty-five percent (154 of 176) of the intracellular recordings were obtained from d.r.g. at segments L_2, L_3 and L_4. The remainder (22 of 176) were obtained from d.r.g. at T_{13} and L_1. The location and numbers of impalements at these different levels reflected, in general, the origin of the sympathetic efferent outflow to the i.m.g. and large intestine. It has been shown that ventral roots at segment L_3 provide the major outflow of sympathetic preganglionic fibres to the i.m.g. (see Dalsgaard & Elfvin, 1979; Krier *et al.* 1982).

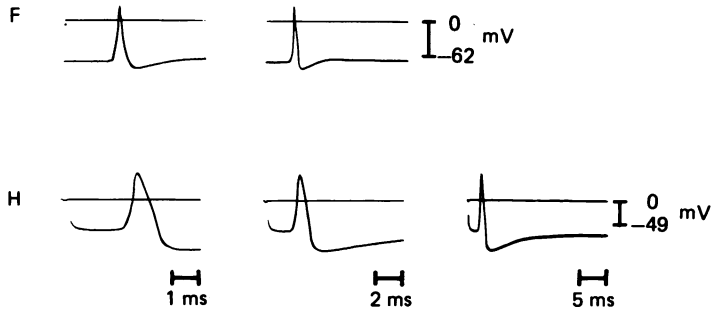

Fig. 3. Action potentials recorded intracellularly and at decreasing (left to right) time bases from two d.r.g. cells showing either a fast or delayed rate of decay during repolarization. Cells were classified as F and H cells accordingly (see text).

Hence, most recordings were made from d.r.g. at L_3 and immediate spinal levels to L_3. On the other hand d.r.g. at segment T_{13}, a level where ventral roots supply few preganglionic fibres, received scant attention.

Following either electrical stimulation of dorsal root filaments (and hence the centripetal axonal branch of a d.r.g. cell) or injection of depolarizing current through the recording micro-electrode, neurones supported an action potential which averaged $82 \pm 1{\cdot}0$ mV ($n = 129$) in amplitude. Since there was an appreciable range in amplitude (60–105 mV), d.r.g. sensory neurones could not be classified confidently as either 'A-ganglion cells' (with A nerve fibres) or 'C-ganglion cells' (with C nerve fibres) as suggested by Bessou, Burgess, Perl & Taylor (1971). However, two populations of neurones could be distinguished by the time-to-decay to half-amplitude ($T\frac{1}{2}$) for the action potential (Fig. 3). For some cells $T\frac{1}{2}$ was short, $0{\cdot}74 \pm 0{\cdot}07$ ms ($n = 13$), while for others it was noticeably longer, $2{\cdot}16 \pm 0{\cdot}20$ ms ($n = 6$). In these latter neurones, there was a distinct plateau during repolarization. This distinction corresponded well to the 'F' (fast) and 'H' (hump) classification for murine sensory neurones (Yoshida, Matsuda & Samejima, 1978). Eighty-two of 176 neurones were classed as F cells while 41 of 176 were identified as H cells. (The remainder (53 of 176) were not classed at the time of recording.) Action potentials were followed by an after-hyperpolarization (a.s.h.). Both amplitude and rate of decay of the a.s.h. depended on cell class (Table 1). Long after-hyperpolarizations in excess of 100 ms as seen with 'A' classed sensory neurones (Yoshida *et al.* 1978) were not observed in this study.

D.r.g. cells displayed unusual electrical properties and showed time-dependent

TABLE 1. Active and passive electrical properties of F- and H-classed sensory neurones. Results are expressed as means ± S.E. of means (n); n.s., not significant, $P \geqslant 0.05$

	F	H	Difference
Resting membrane potential (mV)	−54 ± 0·7 (82)	−47 ± 0·9 (41)	$P \leqslant 0.01$
Characteristics of action potential			
Amplitude (mV)	81 ± 1·3 (70)	82 ± 1·9 (34)	n.s.
After-spike hyperpolarization (mV)	12 ± 0·5 (63)	14 ± 0·9 (32)	$P \leqslant 0.05$
Time-to-decay to half-amplitude (ms)	0·74 ± 0·07 (13)	2·16 ± 0·2 (6)	$P \leqslant 0.01$
Time-to-decay by 90% (ms)	4·19 ± 0·63 (11)	10·4 ± 2·3 (6)	$P < 0.01$
Input resistances			
Input resistance with $I_{depol.}$ (MΩ)	4·2 ± 0·8 (5)	10·6 ± 2·9 (3)	$P \leqslant 0.02$
Input resistance with $I_{hyperpol.}$ (MΩ)	10·9 ± 1·6 (8)	41·7 ± 6·9 (9)	$P \leqslant 0.01$
Threshold current for rectification to $I_{hyperpol.}$ (nA)	1·7 ± 0·2 (6)	0·47 ± 0·04 (3)	$P \leqslant 0.05$

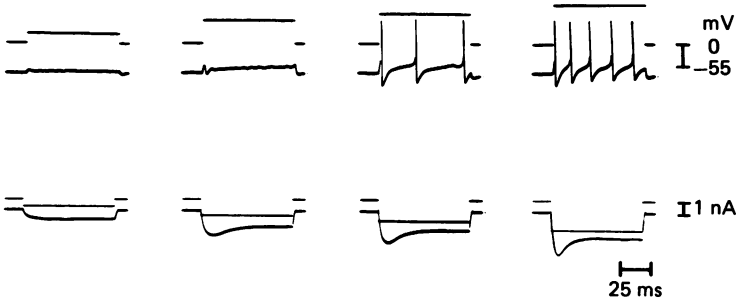

Fig. 4. Time-dependent rectification of potential changes in response to depolarizing (upper row) and hyperpolarizing (lower row) currents applied through an intracellular electrode.

rectification to depolarizing and hyperpolarizing currents injected into the cell via the micro-electrode. With depolarizing current, the voltage trajectory in some cells (40 of 176) showed a sharp depolarization which then relaxed to a less positive steady-state potential (Fig. 4). Both F and H cells displayed this property. The input resistance (R_{in}) of F and H cells, calculated from the slopes of the relationship of steady-state potentials to depolarizing currents, was 4·2 ± 0·8 MΩ ($n = 5$) and 10·6 ± 2·9 MΩ ($n = 3$), respectively. This unusual behaviour to depolarizing current has been shown to be due to a fast-inactivating potassium current (sometimes referred to as I_A) which limits membrane excitability and restricts repetitive firing in d.r.g. cells (Kostyuk, Veselovsky, Fedulova & Tsyndrenko, 1981*b*), in paravertebral sympathetic ganglion cells (Galvan, 1982) and in *Aplysia* neurones (Shimahara, 1981). With hyperpolarizing current, the voltage trajectory in some cells (45 of 176) showed an initial fall in voltage which relaxed slightly to a more positive steady-state potential (Fig. 4). Both F and H cells displayed this property. Input resistance, calculated from the slopes of the relationship of steady-state potentials to hyperpolarizing current, was 10·9 ± 1·6 MΩ ($n = 8$) and 41·7 ± 6·9 MΩ ($n = 9$) respectively for F and H cells. The current threshold for this time-dependent rectification was

$1{\cdot}57 \pm 0{\cdot}2$ nA ($n = 6$) and $0{\cdot}47 \pm 0{\cdot}04$ nA ($n = 3$) respectively for F and H cells. Rectification became obvious at voltages in excess of -80 mV, a value close to the equilibrium potential for potassium (E_K) (Kostyuk *et al.* 1981*b*). In addition to this time-dependent rectification, values for R_{in} were greater for F and H cells when calculated for hyperpolarizing rather than depolarizing currents, indicating that these sensory neurones showed anomalous rectification of current.

The above data suggest two populations of sensory neurones in d.r.g. as judged by their active and passive properties. These properties are summarized in Table 1. A similar distinction has been reported for d.r.g. cells in other mammals (Bessou *et al.* 1971; Czéh, Kudo & Kuno, 1977; Yoshida *et al.* 1978).

Input to neurones in d.r.g.

Following electrical stimulation of dorsal roots in forty-three experiments, an action potential was conducted centrifugally (i.e. towards the periphery) to the soma of 65 of 110 neurones. The remaining 55 (of 110) neurones also gave rise to an action potential but in those cases there was no distinct latency between stimulus artifact and upstroke of the action potential. Therefore for those fifty-five neurones, axonal conduction of excitation was ruled out and the wave form attributed to direct stimulation of the spinal ganglion. Since dorsal roots were short (3–5 mm) and the cathodal pole of the stimulating electrode was often within 1 mm of the spinal ganglion, these factors contributed to direct stimulation.

Following electrical stimulation of spinal sympathetic rami in fifteen experiments, an action potential was conducted centripetally (i.e. towards the spinal cord) to the soma of twelve of fifty-four neurones. However, some of these cells may be renal sensory neurones which are known to enter the cord at the spinal levels tested (Kuo, deGroat, Nadelhaft, Hisamitsu & Backes, 1982). The latency between stimulus and excitation fell into a range 0·3–0·9 ms. Conduction distances were less than 10 mm but could not be measured accurately since the sympathetic rami curve round the spinous processes on lumbar vertebrae. Conduction velocities were therefore in a range of 1–3 m/s, indicative of either small Aδ fibres or C fibres. Those d.r.g. cells which did not respond with an action potential following stimulation of spinal sympathetic rami were considered to be somatic sensory neurones. In support of this view, stimulation of somatic fibres in the lumbar-sacral nerve plexus (in two experiments) evoked neuronal discharge.

Following electrical stimulation of any nerve peripheral to the sympathetic paravertebral chain, i.e. left and right hypogastric, lumbar-colonic, intermesenteric, coeliac and lumbar-splanchnic nerves, none of 176 neurones in forty-three preparations showed an invading action potential. Also, none of the sensory neurones were excited when the distal colon was distended with air to excite intramural mechanoreceptors. These results raised two points concerning the referral of sensory information from the colon. First, mechanoreceptor information from the distal colon may be referred exclusively to prevertebral ganglia to form peripheral reflex arcs with sympathetic neurones therein. Alternatively, sampling from sensory neurones in spinal ganglia with single cell recordings was a highly randomized process and required more observations than made to reveal a small population of mechanoreceptor sensory perikarya. To explore these interpretations, extracellular recording techniques were employed to trace sensory nerve pathways from the gut to the spinal cord.

Extracellular recording from spinal nerve roots

Extracellular recordings were made on dorsal and ventral roots (at the levels of L_1–L_4) in an attempt to monitor multiunit discharge synchronous with electrical stimulation of each of the lumbar-colonic, left hypogastric, intermesenteric and coeliac nerves separately. Again, intramural mechanoreceptors were stimulated also by distending the colon with air. With extracellular electrodes on the dorsal roots, multiunit discharge was never detected in response to either stimulation of peripheral nerves or colonic distension (six experiments). The same held when recordings were made from ventral roots: multiunit discharge was not detected (six experiments). These results suggested an absence or paucity of gut mechanoreceptor C fibres in spinal dorsal and ventral nerve roots, at least at the levels of L_1–L_4.

The apparent absence of mechanoreceptor fibres merited more exhaustive investigations in view of recent histological evidence from mapping techniques of axonal projections using True Blue which suggested the presence of some sensory pathways from the distal colon to lumbar segments via the lumbar-colonic nerves and i.m.g. in the guinea-pig (Dalsgaard & Elfvin, 1977, 1982). Therefore, extracellular recordings were made on lumbar-colonic nerves, the most likely pathway for sensory afferents from the colon to the spinal cord, in the hope of tracing those sensory fibres shown histologically by Dalsgaard & Elfvin (1977, 1982).

Extracellular recording from lumbar-colonic nerves

Recording electrodes were placed on lumbar-colonic nerves several millimetres peripheral to the i.m.g., and stimulating electrodes positioned on either dorsal or ventral roots, at the levels of L_1–L_4. Stimulation of dorsal roots (L_1 d.r.–L_4 d.r.) failed to evoke multiunit discharge from lumbar-colonic nerves (six experiments). Although there is histological evidence of retrograde axonal transport of HRP from the i.m.g. to d.r.g. particularly at the level of L_2 (see '*Application of HRP*' below; also Dalsgaard & Elfvin, 1977, 1982), these fibres represent only a fraction (less than 5%) of sensory afferents and possibly escaped detection by our electrophysiological means. Since they have been implicated with substance P and nociception (Dalsgaard & Elfvin, 1982; Dun & Jiang, 1982) they may be A fibres and not C fibres which carry mechanoreceptor information.

Stimulation of ventral roots (L_2 v.r.–L_4 v.r.) consistently evoked multiunit discharge from lumbar-colonic nerves (six experiments) (Fig. 5). Stimulating ventral roots at the level of L_1 always failed to evoke any response. Stimulation of lumbar-splanchnic nerves to recruit all nerve fibres connected to lumbar spinal ventral roots also gave rise to multiunit discharge from the lumbar-colonic nerves (Fig. 5). Excitation appeared to be conducted unidirectionally because hexamethonium (5×10^{-4} M) interrupted transmission between ventral roots (L_2 v.r.–L_4 v.r.) and the lumbar-colonic nerves and between lumbar-splanchnic and lumbar-colonic nerves (Fig. 5). Therefore all nerves were considered preganglionic fibres, with the i.m.g. presumably the location of the nicotinic synapse. These experiments exclude the possibility of mechanoreceptor C fibres entering the spinal cord via ventral roots.

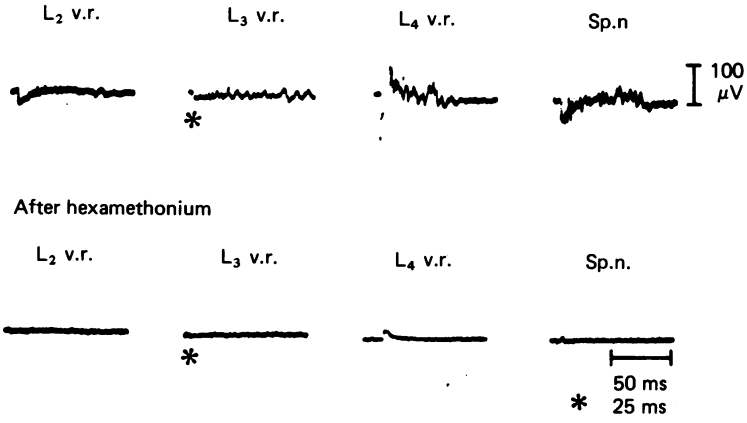

Fig. 5. Multiunit discharge recorded from lumbar-colonic nerve following stimulation of central nerves before (upper row) and after (lower row) exposure of nerves to hexamethonium (5×10^{-4} M). Spinal level of ventral roots is indicated. Changes in time base are indicated by asterisks.

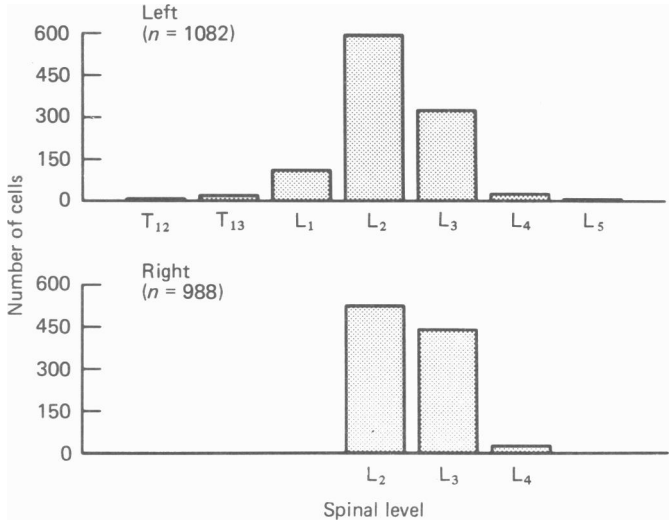

Fig. 6. Distribution and location of sensory perikarya containing HRP.

Application of HRP

HRP was applied to lumbar-colonic nerves, and serial sections (50 μm) from d.r.g. at the levels of T_{12}–T_{13} and L_1–L_5 were processed in the attempt to demonstrate retrograde axonal transport of HRP to these spinal levels. Labelled cell bodies were seen in *left* d.r.g. at the spinal levels of T_{12}–T_{13} and L_1–L_4, but were absent at the level of L_5. The distribution and numbers of HRP-containing perikarya are shown in Fig. 6. Labelled cells were also found in *right* d.r.g. at spinal levels which were investigated histologically, namely L_2–L_4. Their distribution and number are also given (Fig. 6). These distributions showed that there was almost equal numbers of sensory pathways to left and right sides of the spinal cord and to any one spinal level.

Most HRP-positive cells (1885 of 2070) were seen at the levels of L_2 (1119 of 2070) and L_3 (766 of 2070) with the remainder in d.r.g. at the other lumbar and thoracic levels mentioned. This distribution and these numbers are almost identical to values given in other recent studies (Dalsgaard & Elfvin, 1977, 1982). From those reports, total cell numbers for d.r.g. at spinal segment L_2 represented somewhere between 1 and 5% of perikarya present. (This sparse distribution is also seen on Pl. 1.) This fraction may have been too small for detection by intracellular and extracellular recording techniques. Also, the distribution for these sensory neurones may indicate levels of referral of noxious stimuli rather than mechanoreceptor information. In support of this suggestion, studies of gut viscerotomes showed nociceptive fibres from the distal colon to enter the spinal cord at these lumbar levels (Hazarika, Coote & Downman, 1964).

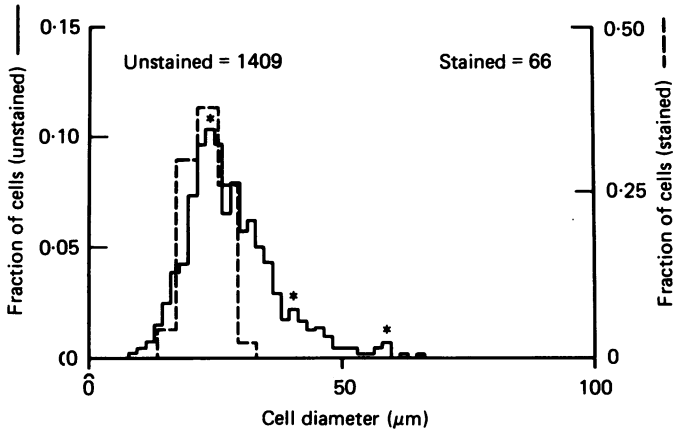

Fig. 7. Distribution of cell sizes for both unstained (continuous line) and stained (dashed line) d.r.g. cells treated for HRP. Asterisks highlight three possible modes at 25, 40 and 60 μm in the distribution of sizes for unstained cells. The positions of these asterisks are virtually identical for the three modes (30, 41 and 65 μm) of cell sizes for lumbar d.r.g. cells observed by Kawamura & Dyck (1978).

The diameter of HRP-containing cells showed an unimodal distribution over a range of 13–34 μm with a mode of 25 μm (Fig. 7). Unstained cells showed a distribution ranging from 11 to 70 μm, with a distinct peak at 25 μm, and perhaps second and third peaks at 40 and 60 μm, respectively (see Fig. 7). Other morphometric studies of lumbar (L_5) ganglia have also shown a similar trimodal distribution of cell diameters (Kawamura & Dyck, 1978). In that study, the first peak appeared at 30 μm, with divisions between successive peaks at 41 and 65 μm. It is difficult to compare results since respective studies were performed on different spinal levels, in our case L_2. The distributions are, however, similar. Since cell diameter is reflective of axon diameter, the cells containing HRP have by inference the smallest fibre diameter of sensory neurones. This would implicate, but not confirm, C fibres. These small fibres may refer nociception, as mentioned above, C fibres being known to carry pain sensation from abdominal viscera to the C.N.S. (Clifton, Coggeshall, Vance & Willis, 1976). Bessou *et al.* (1971) have shown that about 48% of all perikarya in lumbar

spinal ganglia have non-myelinated peripheral axons and are cutaneous sensory neurones. The unstained cells probably were cutaneous sensory neurones. The HRP-containing neurones probably were involved in pain referral.

DISCUSSION

Electrophysiological evidence presented in this study supports the view that sensory information generated by stretch mechanoreceptors in the distal colon was referred mainly, if not exclusively, to the prevertebral ganglia. Using either intracellular or extracellular recording techniques afferent C fibres which conveyed mechanoreceptor information could be traced to the i.m.g. but no further centrally. There appeared to be an absence or extreme paucity of mechanoreceptor C fibres in sympathetic rami and of gut mechanoreceptor perikarya in d.r.g. Our histological evidence, however, indicates that some (2070) sensory fibres in the lumbar-colonic nerves managed to course to the spinal cord, prediominantly to d.r.g. at the level of L_2. This spinal level of entry is similar to the level of entry for representation of pain from colonic viscerotomes (Hazarika *et al.* 1964). Also, the number (2070) of fibres involved is close to the value of 2500 sensory fibres reported to be present in lumbar splanchnic nerves (Harris, 1943). We suggest, therefore, that those fibres which transported HRP in our study were perhaps involved with nociception. We recognize that our sampling number of successfully recorded neurones in the d.r.g. was small relative to the total number of cells contained in each spinal ganglion. A small fraction of gut mechanoreceptor C fibres may have escaped detection, either when recording intracellularly from sensory neurones in d.r.g. or when recording extracellularly from dorsal spinal roots.

The view that the 'receptor element' in the autonomic (sympathetic) reflex arc need not necessarily run to the spinal cord is consistent with previously documented evidence. Kuntz and his colleagues (Kuntz & Van Buskirk, 1941; Kuntz & Saccamano, 1944) found that low-threshold stretch stimuli recruited mechanoreceptor C fibres which projected to the coeliac plexus. Furthermore, the Sokownin reflex (Sokownin, 1874) which involves the i.m.g. remains functional after decentralization, a finding which has been repeatedly confirmed (Langley & Anderson, 1894; Job & Lundberg, 1952; Skok, 1973). Garry (1933) showed that dorsal rhizotomy together with ablation of the spinal cord above L_1 and below L_5 failed to abolish sympathetic tone in the distal colon; however, tone was partially reduced following decentralization and totally abolished only by ganglionectomy. The effect of decentralization seen by Garry (1933) has been interpreted as removal of a tonic efferent input from oscillatory preganglionic B fibres, and ganglionectomy as destruction of peripheral reflex pathways through the prevertebral ganglia (deGroat, Booth, Krier, Milne, Morgan & Nadelhaft, 1979). The functional independence of the cat i.m.g. to maintain sympathetic tone following decentralization is also shared by that of the dog (Lawson & Holt, 1938). This may be due to the dog i.m.g. receiving a peripheral input from C fibres in the lumbar-colonic as well as hypogastric and intermesenteric nerves (King & Szurszewski, 1984). The rabbit i.m.g. may also participate in some peripheral reflex pathways (Brown & Pascoe, 1952). This ganglion receives mechanoreceptor input from the distal colon (Kreulen, 1982). In all these mammalian prevertebral ganglia

mentioned, mechanoreceptor input to sympathetic neurones involved cholinergic transmission. Notably, acetylcholine and the enzyme choline acetylase are virtually absent in perikarya of d.r.g. (Feldberg & Vogt, 1948; Hebb, 1955; Hebb & Silver, 1956), further suggesting the absence of gut mechanoreceptor cholinergic sensory neurones in spinal ganglia. Thus, the data reported in the present study as well as data previously reported by others strongly suggest that gut mechanoreceptor information is mainly if not exclusively referred to the prevertebral ganglia.

Our data on the active and passive electrical properties of d.r.g. sensory neurones suggested that there were two populations of cells. For convenience, these populations of cells were recognized as H and F cells, after the broad classification of Yoshida *et al.* (1978). Bessou *et al.* (1971) also classified sensory neurones into two groups (A and C cells) on the basis of nerve fibre conduction velocity. The data obtained in the present study provides evidence to link the C cell of one classification with the H cell of the other classification. In this study, sensory neurones which showed input following stimulation of sympathetic rami gave rise to an action potential with a distinct plateau during repolarization, i.e. H cells. A recent investigation showed that action potential duration in rat d.r.g. cells is related to conduction velocity (Harper & Lawson, 1982). In our case cells with a distinct plateau during repolarization showed fibre conduction velocities indicative of C fibres. If H cells correspond to C cells, the question arises as to whether or not F cells are analogous with A cells. By definition, A cells are cutaneous sensory neurones (Bessou *et al.* 1971). If F cells observed in our studies are also cutaneous sensory cells, then they are not involved with referral of sensory information by gut mechanoreceptors.

The electrical properties of H and F d.r.g. cells showed time-dependent rectification of voltage to hyperpolarizing current pulses. This phenomenon has been observed previously for other mammalian d.r.g. cells (Czéh *et al.* 1977; Yoshida *et al.* 1978) although the mechanisms underlying this response were not fully understood. Recently, Kostyuk, Veselovsky & Fedulova (1981*a*) and Kostyuk *et al.* (1981*b*) showed that d.r.g. cells possessed two distinct components, fast and slow, to the potassium current. The fast component $I_{f,K}$ was inactivated rapidly at holding potentials more positive than -50 mV, a value close to the resting membrane potential reported here. However, $I_{f,K}$ was activated fully at more negative (-120 mV) holding potentials. Therefore, the channels responsible for carrying this current will be brought into action during membrane hyperpolarization and the subsequent increase in membrane conductance (decrease in membrane resistance) may well account for the relaxation of voltage seen with hyperpolarizing currents. Although H and F cells displayed this time-dependent rectification, the threshold current was different for the two classes of cells. This difference may be related, in part, to cell size (Jack, Noble & Tsien, 1975). F cells which required a greater threshold current for rectification may be larger in size, a greater applied current being necessary to maintain current density to produce at given voltage. From Pl. 1, it was evident that cell size was not homogeneous and, following measurements of cell diameters, a trimodal distribution of size could possibly be inferred. If F cells are larger, this may add further weight to the suggestion that they are indeed A-type ganglion cells. Their lower input resistances would also support the contention that F cells are larger.

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REFERENCES

ADAMS, J. C. (1977). Technical considerations on the use of horseradish peroxidase as a neuronal marker. *Neuroscience* **2**, 141–145.

ARCHAKOVA, L. I., BULYGIN, I. A. & NETUKOVA, N. I. (1982). The ultrastructural organization of sympathetic ganglia of the cat. *J. auton. nerv. Syst.* **6**, 83–93.

BESSOU, P., BURGESS, P. R., PERL, E. R. & TAYLOR, C. B. (1971). Dynamic properties of mechanoreceptors with unmyelinated (C) fibers. *J. Neurophysiol.* **34**, 116–131.

BROWN, G. L. & PASCOE, J. E. (1952). Conduction through the inferior mesenteric ganglion of the rabbit. *J. Physiol.* **118**, 113–123.

CHANG, P. Y. & HSU, F. Y. (1942). The localisation of the intestinal inhibitory reflex arc. *Q. Jl exp. Physiol* **31**, 311–318.

CLIFTON, G. L., COGGESHALL, R. E., VANCE, W. H. & WILLIS, W. D. (1976). Receptive fields of unmyelinated ventral root afferent fibres in the cat. *J. Physiol.* **256**, 573–600.

CROWCROFT, P. J., HOLMAN, M. E. & SZURSZEWSKI, J. H. (1971). Excitatory input from the distal colon to the inferior mesenteric ganglion in the guinea-pig. *J. Physiol.* **219**, 443–461.

CZEH, G., KUDO, N. & KUNO, M. (1977). Membrane properties and conduction velocity in sensory neurones following central or peripheral axotomy. *J. Physiol.* **270**, 165–180.

DALSGAARD, C. J. & ELFVIN, L. G. (1977). Retrograde axonal transport of horseradish peroxidase in afferent fibers of the inferior mesenteric ganglion of the guinea-pig. Identification of cells of origin in dorsal root ganglia. *Brain Res.* **126**, 149–153.

DALSGAARD, C. J. & ELFVIN, L. G. (1979). Spinal origin of preganglionic fibers projecting onto the superior cervical ganglion and inferior mesenteric ganglion of the guinea pig, as demonstrated by the horseradish peroxidase technique. *Brain Res.* **172**, 139–143.

DALSGAARD, C. J. & ELFVIN, L. G. (1982). Structural studies on the connectivity of the inferior mesenteric ganglion of the guinea-pig. *J. auton. nerv. Syst.* **5**, 265–278.

DE GROAT, W. C., BOOTH, A. M., KRIER, J., MILNE, R. J., MORGAN, C. & NADELHAFT, I. (1979). Neural control of the urinary bladder and large intestine. In *Integrative Functions of the Autonomic Nervous System*, ed. BROOKS, C. MC C., KOIZUMI, K. & SATO, A., chap. 4, pp. 50–67.

DE GROAT, W. C. & KRIER, J. (1979). The central control of the lumbar sympathetic pathway to the large intestine of the cat. *J. Physiol.* **289**, 449–468.

DUN, W. J. & JIANG, Z. G. (1982). Non-cholinergic excitatory transmission in inferior mesenteric ganglia of the guinea-pig: possible mediation by substance P. *J. Physiol.* **325**, 149–159.

FELDBERG, W. & VOGT, M. (1948). Acetylcholine synthesis in different regions of the central nervous system. *J. Physiol.* **107**, 372–381.

GALVAN, M. (1982). A transient outward current in rat sympathetic neurones. *Neurosci. Lett.* **31**, 295–300.

GARRY, R. C. (1933). The nervous control of the caudal region of the large bowel. *J. Physiol.* **77**, 422–431.

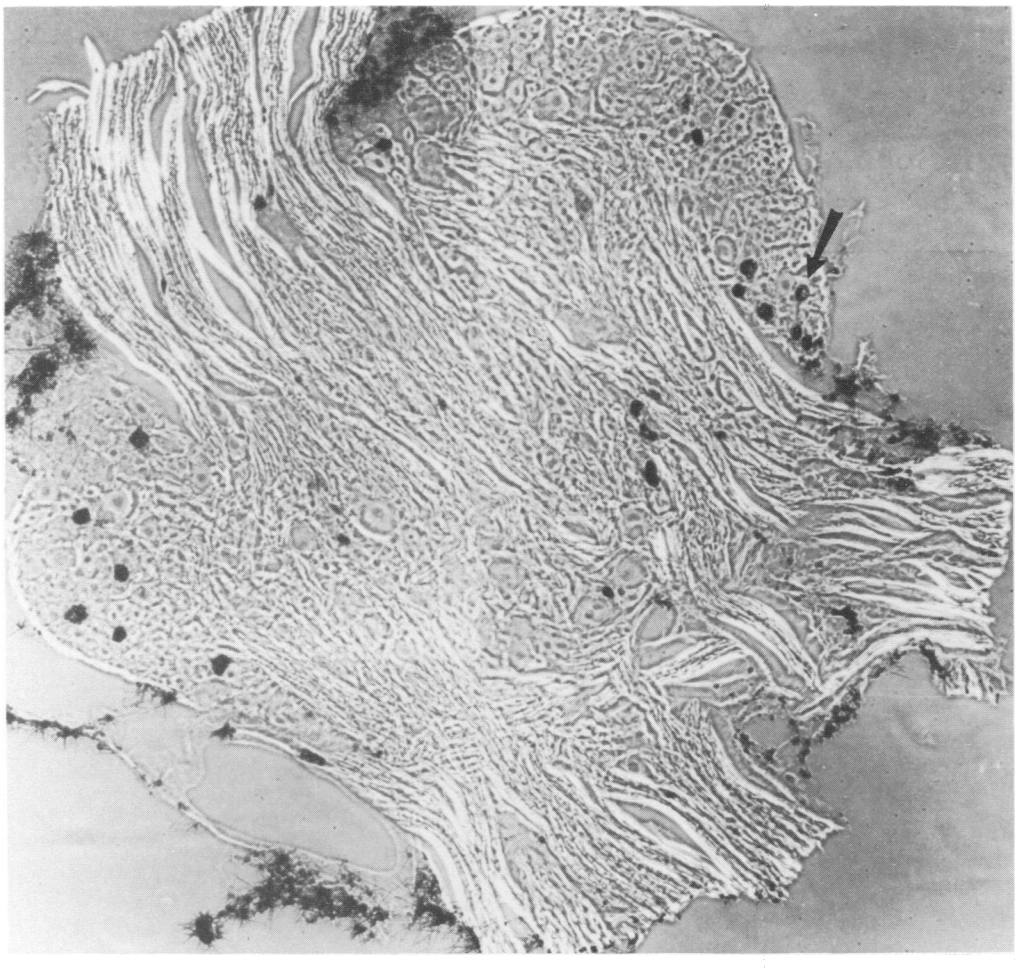
HARPER, A. A. & LAWSON, S. N. (1982). Action potential duration, axonal conduction velocity and cell size in rat dorsal root ganglion neurones. *J. Physiol.* **332**, 22–23*P*.

HARRIS, A. J. (1943). An experimental analysis of the inferior mesenteric plexus. *J. comp. Neurol.* **79**, 1–17.

HAZARIKA, N. H., COOTE, J. & DOWNMAN, B. C. B. (1964). Gastrointestinal dorsal root viscerotomes in the cat. *J. Neurophysiol.* **27**, 107–116.

HEBB, C. O. (1955). Choline acetylase in mammalian and avian sensory systems. *Q. Jl exp. Physiol.* **40**, 176–186.

HEBB, C. O. & SILVER, A. (1956). Choline acetylase in the central nervous system of man and some other mammals. *J. Physiol.* **134**, 718–728.



JACK, J. J. B., NOBLE, D. & TSIEN, R. W. (1975). Influence of fibre size on excitation threshold. In *Electrical Current Flow in Excitable Cells*, p. 276. Oxford: Clarendon Press.

JOB, C. & LUNDBERG, A. (1952). Reflex excitation of cells in the inferior mesenteric ganglion on stimulation of the hypogastric nerve. *Acta physiol. scand.* **26**, 366–382.

KAWAMURA, Y. & DYCK, P. J. (1978). Evidence for three populations by size in L_S spinal ganglia in man. *J. Neuropath. exp. Neurol.* **37**, 269–272.

KING, B. F. & SZURSZEWSKI, J. H. (1984). An electrophysiological study of the inferior mesenteric ganglion of the dog. *J. Neurophysiol.* (in the Press).

KOSTYUK, P. G., VESELOVSKY, N. S. & FEDULOVA, S. A. (1981*a*). Ionic currents in the somatic membrane of rat dorsal root ganglion neurones. II. Calcium currents. *Neuroscience* **6**, 2431–2438.

KOSTYUK, D. G., VESELOVSKY, N. S., FEDULOVA, S. A. & TSYNDRENKO, A. Y. (1981*b*). Ionic currents in the somatic membrane of rat dorsal root ganglion neurons. III. Potassium currents. *Neuroscience* **6**, 2439–2444.

KREULEN, D. L. (1982). Properties and synaptic response of the inferior mesenteric ganglion of the rabbit. *Physiologist* **25**, 313.

KREULEN, D. L. & SZURSZEWSKI, J. H. (1979). Reflex pathways in the abdominal prevertebral ganglia. Evidence for a colocolonic inhibitory reflex. *J. Physiol.* **295**, 21–32.

KRIER, J., SCHMALZ, P. F. & SZURSZEWSKI, J. H. (1982). Central innervation of neurones in the inferior mesenteric ganglion and of the large intestine of the cat. *J. Physiol.* **332**, 125–138.

KUNTZ, A. & VAN BUSKIRK, C. (1941). Reflex inhibition of bile flow and intestinal motility mediated through decentralized celiac plexus. *Proc. Soc. exp. Biol.* **46**, 519–523.

KUNTZ, A. & SACCAMANO, G. (1944). Reflex inhibition of intestinal motility mediated through decentralised prevertebral ganglia. *J. Neurophysiol.* **7**, 163–170.

KUO, D. C., DEGROAT, W. C., NADELHAFT, I., HISAMITSU, T. & BACKES, M. G. (1982). The origin of renal sympathetic efferents, and the central projection of renal afferents in cat. *Neurosci. Abstr.* **8**, 79.

LANGLEY, J. N. & ANDERSON, H. K. (1894). On reflex action from sympathetic ganglia. *J. Physiol.* **16**, 410–440.

LAWSON, H. & HOLT, J. P. (1938). The control of the large intestine by the decentralized inferior mesenteric ganglion. *Am. J. Physiol.* **118**, 780–785.

NJÅ, A. & PURVES, D. (1977). Specific innervation of guinea-pig superior cervical ganglion cells by preganglionic fibres arising from different levels of the spinal cord. *J. Physiol.* **264**, 565–583.

OFFORD, K., OHTA, M., EONNING, R. F. & DYCK, P. J. (1974). Method of morphometric evaluation of spinal and autonomic ganglia. *J. neurol. Sci.* **22**, 65–71.

ROSS, J. G. (1954). On the presence of centripetal fibres in the superior mesenteric nerves of the rabbit. *J. Anat.* **92**, 189–197.

SHIMAHARA, T. (1981). Modulation of synaptic output by the transient outward current in *Aplysia*. *Neurosci. Lett.* **24**, 139–142.

SKOK, V. I. (1973). *Physiology of Autonomic Ganglia*, 1st edn., p. 43, Tokyo: Igaku, Shoin Ltd.

SOKOWNIN, N. (1874). Cited by Kowalewsky, N. & Arnstein, C. *Pflügers Arch. ges. Physiol.* **8**, 600.

SZURSZEWSKI, J. H. & WEEMS, W. A. (1976). A study of peripheral input to and its control by post-ganglionic neurones of the inferior mesenteric ganglion. *J. Physiol.* **256**, 541–556.

UNGÁVY, G. & LÉRÀNTH, C. (1970). Termination in the prevertebral abdominal sympathetic ganglia of axons arising from the local (terminal) regulative plexus of visceral organs. *Z. Zellforsch. mikrosk. Anat.* **111**, 185–191.

YOSHIDA, S., MATSUDA, Y. & SAMEJIMA, A. (1978). Tetrodotoxin-resistant sodium and calcium components of action potentials in dorsal root ganglion cells of the adult mouse. *J. Neurophysiol.* **41**, 1096–1106.

EXPLANATION OF PLATE

Montage of photomicrographs showing a section of a dorsal root ganglion at the level of the second lumbar segment (L_2) treated to develop for HRP. The dark aggregates (see arrow) within the section show perikarya which accumulated HRP following retrograde axonal transport of the substance from the lumbar-colonic nerves. Some non-specific staining occurred outwith the section. Viewed using phase-constrast microscopy. Magnification 100×.