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THE INTRAMURAL PELVIC NERVES IN THE COLON OF DOGS

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SUMMARY

1. The intramural pelvic nerves of the dog colon were studied morphologically and electrophysiologically.

2. These nerves emanate from the pelvic plexus, and ascend between the muscle layers along 57·5 % of the colon's length. Many thin branches connect with Auerbach's plexus.

3. Most of the nerves responded bilaterally with compound action potentials of Aδ and C fibres to stimulation of the sacral dorsal roots, and with those of C fibres to stimulation of the ventral roots. Mean conduction velocities of the Aδ and C fibres were 9·0 and 0·9 m/s, respectively.

4. Distension of a section of colon at up to 85·0 % of the length of the colon from the anus activated afferent fibres, and electrical stimulation of points at up to 102 % of this length elicited responses in efferent fibres.

5. In dogs in which the continuity of the colonic wall was interrupted by a ligature but the nerves were left intact, the intramural pelvic nerves were found to convey centrifugal activities of the recto- and ano-colonic reflexes to the proximal colon, and centripetal and centrifugal activities of the colo-colonic reflex.

INTRODUCTION

It is well established that efferent stimulation of the pelvic nerve enhances motility, secretion and blood flow of the distal as well as the proximal colon. Since these effects disappear above a ligature which breaks the continuity of the colonic wall, they are believed to be mediated by nerve fibres in the wall (Jennings, 1938; Hultén, 1969). However, no description of the morphology and physiology of the fibres responsible for these effects is available.

Recently, in dogs, we have found nerve bundles that branch from the pelvic nerve and ascend to the proximal colon between the longitudinal and circular muscle layers. We have termed these nerves the intramural pelvic nerves. The present work aims to elucidate the fibre types in these intramural pelvic nerves, the area they innervate and their role in the control of colonic motility.

METHODS

Preparatory surgery. Thirty-two adult dogs of 6–12 kg and five infant dogs of 0·5–1·0 kg were used in these experiments.

In six adult dogs, the left pelvic, lumbar colonic and bilateral hypogastric nerves were severed under general anaesthesia (25 mg sodium pentobarbitone/kg) and allowed to degenerate for 4–7 weeks. The intramural pelvic nerves of the dogs were subsequently examined with an electron microscope.

In twenty-one adult dogs anaesthetized with α-chloralose (100 mg/kg) and paralysed with gallamine triethiodide (1 mg/kg), the fibre types in the intramural pelvic nerves and the area of the colon they innervate were examined by an electrophysiological method. In four of them, the spinal ganglia of the sacral nerves were excised bilaterally under anaesthesia with sodium pentobarbitone, and the afferent fibres in the sacral nerves and intramural pelvic nerves were allowed to degenerate for 4–7 weeks. The upper limit of innervation of the colon by efferent fibres of the intramural pelvic nerves was determined.

In five dogs decerebrated under anaesthesia with ketamine hydrochloride (10 mg/kg), the role of the intramural pelvic nerves in the reflex control of colonic motility was examined.

Histological methods. The intramural pelvic nerves of the infant dogs were stained by Schabadasch's method under barbiturate anaesthesia (30 mg sodium pentobarbitone/kg; Schabadasch, 1930). Each dog was perfused from the thoracic aorta with 1 l Tyrode solution, then with 1 l Methylene Blue solution containing 8 g NaCl, 2 g glucose and 0·35 g Methylene Blue per litre. After perfusion the whole colon was removed, cut longitudinally along the mesenteric border, and pinned out on a wooden board in Tyrode solution cooled to about 10 °C. The longitudinal muscle layer and sometimes the mucosa was gently stripped off. The intramural pelvic nerves were stained blue, so they were visible over their entire length.

For observation under the electron microscope, the intramural pelvic nerves were isolated from the distal colon under anaesthesia with α-chloralose (100 mg/kg) and fixed *in situ* with a 1·5 % (w/v) glutaraldehyde and 1·5 % (w/v) formaldehyde mixture in 0·1 M-phosphate buffer (pH 7·4), while these nerves were still physiologically active. Each nerve was then dissected into small pieces and refixed in the same fixative for 3 h at room temperature. A post-fixation was carried out for 2 h at room temperature in a 1 % (w/v) osmium tetroxide solution, adjusted to pH 7·4 with 0·1 M-phosphate solution. After fixation, tissue blocks were dehydrated through a graded ethanol series and embedded in epoxy resin. Thin sections cut on an ultramicrotome were doubly stained with 2% uranyl acetate and Reynolds lead citrate solutions and examined under an electron microscope.

Electrophysiological method. The colon was exposed by a mid-line incision and removal of the jejunum and ileum. The lumbar colonic nerve, hypogastric nerves and the nerves along the middle, left and right colic arteries were severed. The intramural pelvic nerves were isolated from the colonic wall. In some dogs, the ventral and dorsal roots of the three sacral nerves were exposed by laminectomy (L4–S3), and stimulated with the dog lying on its right side. In these experiments, the colon was exposed by excision of the left abdominal wall. The colon and isolated nerves were covered with mineral oil.

Responses of the intramural pelvic nerves to electrical stimulation of the colonic wall or roots of the sacral nerves were averaged over 50–100 samples with a digital computer. Centripetal and reflex discharges of the intramural pelvic nerves after distension of the colon and rectum, and mechanical stimulation of the anal mucosa, were fed into spike counters and converted into frequency histograms of 1–4 s bins. For recording and electrical stimulation, bipolar platinum wire electrodes were used. In experiments designed to study the role of the intramural pelvic nerves in the reflex control of colonic motility, all of the nerves were isolated about 2 cm above the junction with the inferior mesenteric artery, and the colon was ligated to break the continuity of the smooth muscle without disturbing the nerves. A wooden block was inserted into the colon to make the ligation more effective. An acrylic resin rod 12 mm in diameter was inserted into the anal canal and rotated manually for mechanical stimulation of the anal mucosa. Motility of the colon and rectum was recorded by the balloon-pressure transducer method. The same balloons were used for distension.

Anaesthetized or decerebrate dogs paralysed with intravenous gallamine triethiodide (1 mg/kg)

were artificially ventilated through a tracheal cannula at a rate of 30–35 strokes/min and a tidal volume of 50–150 ml. Body temperature was maintained at about 36 °C by the heat from two 100 W tungsten lamps.

RESULTS

Histological observations

The intramural pelvic nerves of infant dogs whose entire colons were stained with Methylene Blue were seen to branch from the pelvic plexus and ascend the colonic wall along the outer surface of the circular muscle layer, some of them going beyond where the inferior mesenteric artery connects with the colon (Pl. 1). The nerves divide the meshwork of Auerbach's plexus into strips and none of them bridges over or tunnels beneath the meshwork. Many fibres, too fine to be seen in the photograph, branch off from the nerves and join Auerbach's plexus, so that the nerves gradually become thinner and thinner, and finally disappear when all their fibres have fused into Auerbach's plexus. The intramural pelvic nerves could be traced for a maximum of $57 \pm 2{\cdot}5\,\%$ (mean $\pm$ S.D., $n = 5$) of the colon's length.

Plate 2 shows two examples of electron micrographs of the nerves of dogs in which the left pelvic nerve was severed and had undergone degeneration. As shown in Pl. 2*B*, the intramural pelvic nerves are enveloped in thin layers of sheath cells which, however, do not extend over the fine branches (↑) connecting the nerves with Auerbach's plexus. The presence of both degenerated fibres, indicated by morphologically abnormal Schwann cells (↑), and intact fibres in the intramural pelvic nerves (Pl. 2*A*) demonstrates that the nerves are composed of fibres from the bilateral pelvic nerves.

Fibre types in the intramural pelvic nerves

Fibre types in the intramural pelvic nerves were defined by measuring conduction velocities of their compound action potentials as shown in Fig. 1. The conduction velocity of small peaks was 3·9 m/s (*A* and *B*), which indicates that these peaks represent a group of Aδ fibres. The conduction velocity (0·6 m/s) of the larger peaks in *C* and *D* shows that these peaks represent a group of C fibres. Similar experiments were performed in thirteen dogs, and conduction velocities, which ranged from 28·1 to 0·42 m/s, were calculated from ninety-five peaks. The mean conduction velocity of seventy-four peaks slower than 3 m/s was $0{\cdot}9 \pm 0{\cdot}4$ m/s, and that of the remaining twenty-one peaks was $9{\cdot}0 \pm 6{\cdot}3$ m/s.

The origin of the Aδ and C fibres was sought by observing the responses of one intramural pelvic nerve in each of six dogs to stimulation of the ventral and dorsal roots of the sacral nerves. The nerves responded to stimulation of the dorsal roots first on the left side and then on the right with the following combinations of Aδ and C fibre peaks: Aδ+C and Aδ+C in three nerves; Aδ+C and C in one nerve (Fig. 2); Aδ+C and no response in one nerve, and Aδ and no response in one nerve. Five of the six nerves responded with C fibre peaks to stimulation of the ventral roots of both sides, and one nerve responded with C fibre peaks to stimulation of the right ventral roots, but not to stimulation of the left ones. Thus most of the intramural pelvic nerves consist of Aδ and C afferent fibres, and C efferent fibres which stem bilaterally from

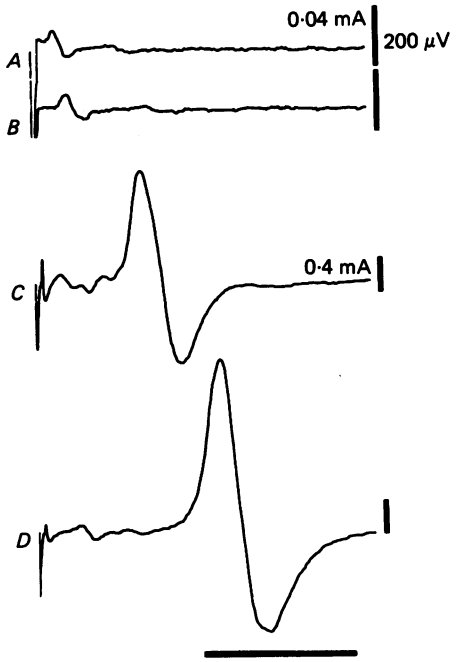

Fig. 1. Compound action potential of an intramural pelvic nerve. The records were taken from the peripheral cut end of an intramural pelvic nerve in response to stimulation of the basal part of the nerve with pulses of 0·1 ms duration, 1 Hz and the intensities indicated. The horizontal bar indicates 2 ms in *A* and *B*, and 10 ms in *C* and *D*.

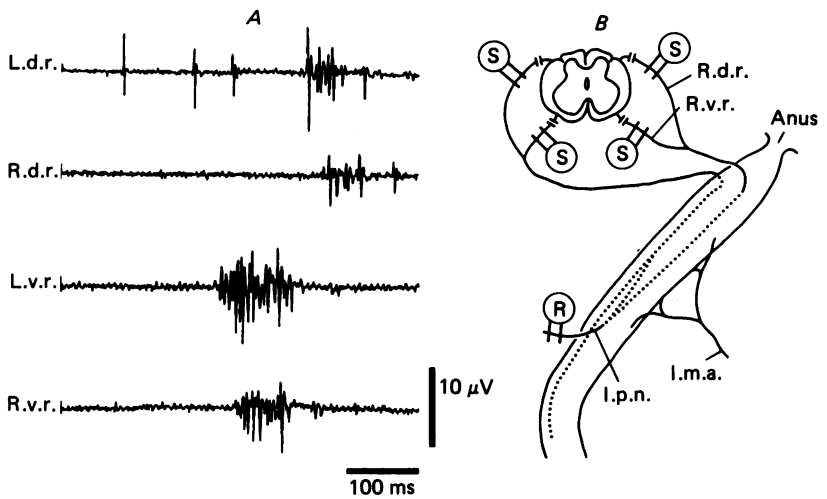

Fig. 2. Responses of an intramural pelvic nerve to stimulation of the ventral and dorsal roots of the sacral cord. The ventral roots of the three sacral nerves were stimulated by pulses of 0·5 ms duration, 1 Hz and 6 V, and the dorsal roots by the same duration and frequency, but 20 V. *A*: from top to bottom the traces show the responses of an intramural pelvic nerve (i.p.n.) to stimulation of the left dorsal roots (l.d.r.), the right dorsal roots (r.d.r.), the left ventral roots (l.v.r.) and the right ventral roots (r.v.r.). The initial three peaks in the response to l.d.r. are of Aδ fibres, and all other peaks are of C fibres. *B*: experimental arrangement. I.m.a., inferior mesenteric artery. S, stimulating electrode. R, recording electrode. The same abbreviations are used in the following Figures.

the sacral cords. The latency of the C fibre peaks in response to stimulation of ventral roots was longer than that of the Aδ fibre peaks, but shorter than that of the C fibre peaks in response to stimulation of the dorsal roots, as shown in Fig. 2. No B fibre peak was observed in response to stimulation of the ventral sacral roots.

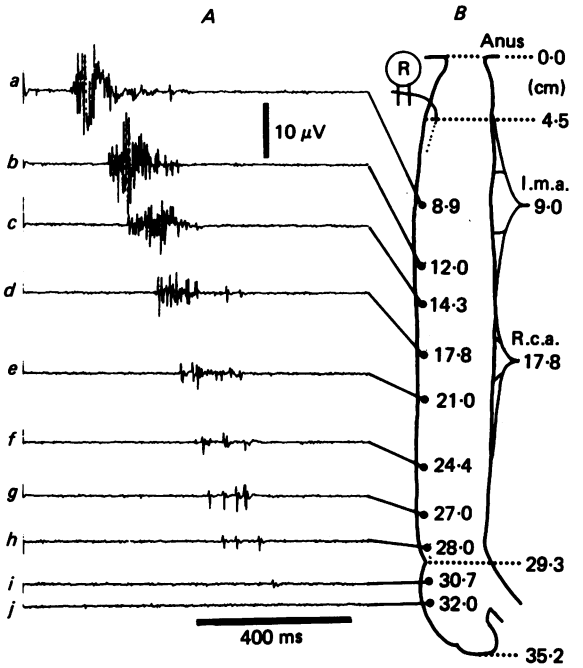

Fig. 3. Responses of an intramural pelvic nerve to stimulation of the colonic wall. The spinal ganglia of all sacral nerves were excised 37 days before this experiment. The experimental arrangement is shown in *B*. Numerals show the distance from the anus. R.c.a., right colic artery. This abbreviation is used in the following Figures. *A*: *a–j* show the responses, which are connected by a line to the point of the colon stimulated. Stimuli, 0·5 ms duration, 0·5 Hz and 10 mA.

Upper limit of innervation of the colon by the intramural pelvic nerves

Four to six intramural pelvic nerves in each dog were exposed at their base, and responses of the peripheral cut ends to stimulation of the colonic wall were examined to determine the upper limits of innervation of the colon by the afferent and efferent fibres.

The upper limit of efferent innervation of the colon by intramural pelvic nerves was studied in dogs in which afferent fibres in the nerve had degenerated. The nerve which yielded recordings shown in Fig. 3 responded with C fibre peaks only. The latency of the responses increased, and the numbers of peaks and their amplitude decreased as the stimulating point was moved away from the anus. This nerve responded with a small peak to stimulation of the caecum 30·7 cm from the anus, but not to stimulation at a point 32·0 cm away, and had the longest reach of five intramural pelvic nerves examined in the same dog. The mean upper limit was $101{\cdot}6 \pm 3{\cdot}9$ % of the length of the colon in this and three other dogs prepared similarly.

The uppermost point of the colon at which afferent fibres of the intramural pelvic nerves responded to distension of the colon was determined (Fig. 4). The discharging frequency recorded from the peripheral cut ends of the nerves was increased by distension of the colon. The uppermost response of those of six other nerves tested in the same dog was evoked by distension of the very proximal end of the colon, even though the responses decreased as the colon was distended more proximally (*d* in Fig. 4). The centre of the distended area was regarded as the upper limit of afferent

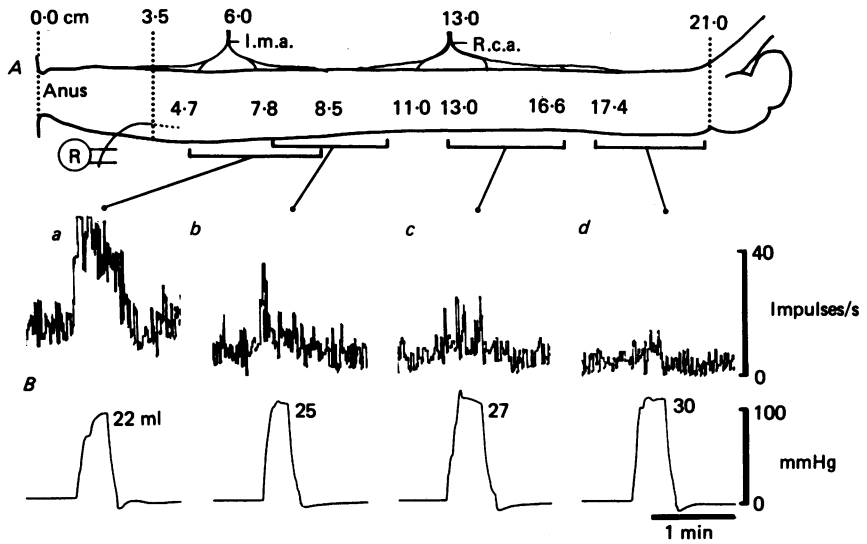

Fig. 4. Responses of an intramural pelvic nerve to distension of the colon. The experimental arrangement is shown in *A*. Numerals show the distance from the anus. *B*: upper trace, discharges of the nerve. Lower trace, intraluminal pressure. *a–d* show the responses, which are connected by a line to the corresponding segment of the colon distended (brackets).

innervation. The mean of the upper limit in five dogs was $85{\cdot}0 \pm 7{\cdot}6$ % of the length of the colon. The upper limit of the afferent innervation of the colon by the nerves was studied further by using a gentle pinch of the colonic wall with large forceps as the stimulus. The mean of the upper limit in seven dogs was $87{\cdot}4 \pm 6{\cdot}2$ % of the length of the colon.

There is a possibility that this method of stimulation activates efferent fibres as well as afferent ones. This possibility was tested in the intramural pelvic nerves of three dogs from which the spinal ganglia of the sacral cord were excised, and accordingly afferent fibres in the nerve had undergone degeneration. The intramural pelvic nerves did not respond to colonic distension of less than 150 mmHg, but did respond to stronger distension. Since the stronger distension may stimulate efferent fibres, we used a distension pressure of less than 120 mmHg to stimulate afferent fibres selectively. Efferent fibres are stimulated quite easily by the pinching of the colonic wall; therefore, we used such a weak pinch that no mark was made on the wall.

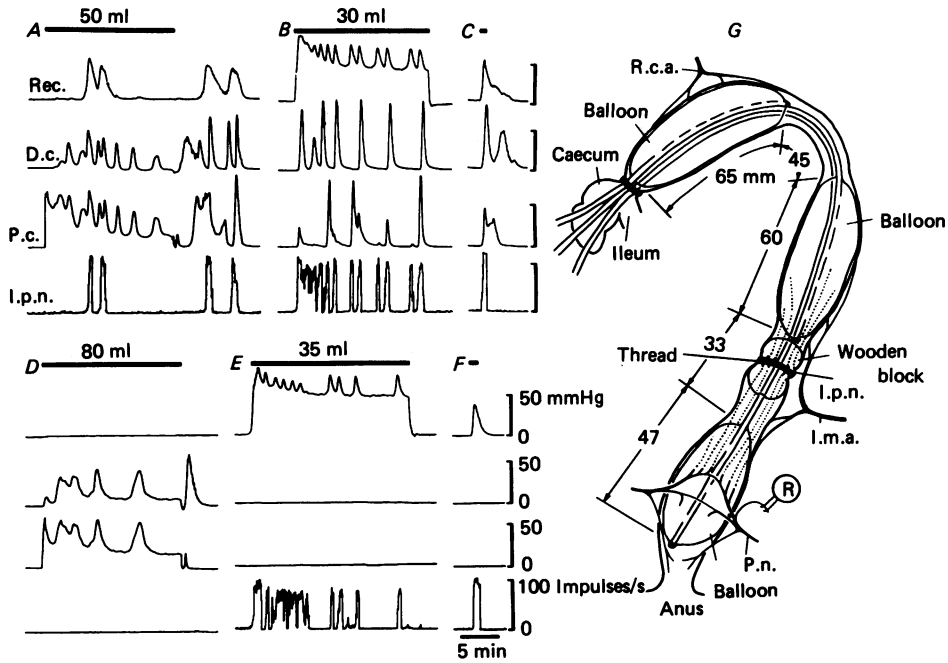

Fig. 5. Effects of distension of the colon and rectum, and mechanical stimulation of the anal mucosa on their motility and the centrifugal activity of the intramural pelvic nerve. The experimental arrangement is shown in *G*. Six of the intramural pelvic nerves were isolated. The colon, but not the isolated nerves, was ligated. One other intramural pelvic nerve was isolated at its base, and the discharges from its central cut end were recorded. Three balloons were used to record the intraluminal pressure of the proximal and distal colon, and of the rectum. Traces: Rec., d.c. and p.c., intraluminal pressure of the rectum, distal colon and proximal colon, respectively. I.p.n., discharges of an intramural pelvic nerve. The proximal colon (*A* and *D*) and the rectum (*B* and *E*) were distended with water of the volumes indicated above each horizontal bar, which show the stimulating periods. The anal musoca (*C* and *F*) was stimulated mechanically. All the isolated intramural pelvic nerves except for the one used for recording were cut between *C* and *D*.

Role of the intramural pelvic nerves in the reflex control of colonic motility

All the extrinsic nerves of the colon other than the pelvic nerves were cut to exclude other reflexes mediated by these nerves. The continuity of the colonic wall was interrupted by a ligature, but the isolated intramural pelvic nerves were left intact. Distension of the proximal colon repeatedly elicited contractions of it and the distal colon, and induced two bursting discharges of the intramural pelvic nerves. Each bursting discharge caused a simultaneous contraction of the whole colon and rectum (Fig. 5*A*). Withdrawal of the water was followed by contraction of the proximal and distal colon, which in turn caused bursting discharges of the nerve and corresponding contractions of the colon and rectum. Distension of the rectum repeatedly elicited bursting discharges and corresponding contractions of the rectum and sometimes of the proximal and distal colon (Fig. 5B). Mechanical stimulation of the anal mucosa elicited bursting discharges and contractions of the colon and rectum (Fig. 5*C*). After all the isolated intramural pelvic nerves were cut, distension of the proximal colon

elicited contractions during and after the distension as before, but did not cause any discharge of the nerve nor any contraction of the rectum (Fig. 5*D*). On the contrary, bursting discharges and corresponding rectal contractions were elicited by distension of the rectum and mechanical stimulation of the anal mucosa; however, the discharge did not cause any contraction of the colon proximal to the ligature (Fig. 5*E* and *F*).

Similar effects of distension of the rectum and mechanical stimulation of the anal mucosa were confirmed in five decerebrated dogs. In these dogs, distension of the colon proximal to the ligature of up to 30 ml did not elicit any discharge of the nerve nor any change in the motility of the rectum distal to the ligature, but distension of 50–100 ml increased efferent discharges of the rectal branch and motility of the rectum.

The above results show that the intramural pelvic nerves mediate the centrifugal activity of the recto- and ano-colonic, and colo-colonic reflexes to the colon, and the centripetal activity from the colon to the sacral cord.

DISCUSSION

Fibres in the intramural pelvic nerves

It was found in this study that some rectal branches of the pelvic nerve ascend between the longitudinal and circular muscular layers of the colon to about 60% of its length. We called these ascending branches the intramural pelvic nerves. The intramural pelvic nerves consist of afferent Aδ and C fibres and efferent C fibres which originate bilaterally from the sacral cord.

The efferent C fibres are thought to be post-ganglionic fibres of the parasympathetic outflow of the pelvic nerve, because most preganglionic fibres in this nerve are B fibres that synapse with the post-ganglionic cells in the pelvic plexus (K. Fukai & H. Fukuda, unpublished observations). This relationship is clearly shown in the recordings of Fig. 2*B*. The latency of the compound action potentials of efferent C fibres due to stimulation of the ventral sacral roots was shorter than that of afferent C fibres elicited by stimulation of the dorsal sacral roots, probably because the action potential of the efferent C fibres is conducted through preganglionic B fibres until it arrives at the post-ganglionic cells in the pelvic plexus.

The possibility that some fibres from the hypogastric nerve mix with the intramural pelvic nerve was not tested fully in this study, but in three dogs pinching of the proximal colonic wall evoked no detectable electrical response in peripheral cut ends of the hypogastric nerves. Results of these experiments showed that all fibres ascending through the intramural pelvic nerves to the proximal colon come from the pelvic nerve.

The area innervated by the intramural pelvic nerves

How the colon is innervated by parasympathetic outflows of both the vagus and pelvic nerves is an old problem that has not been answered fully (Schmidt, 1933).

We found that the entire length of the colon ($101.6 \pm 3{\cdot}4\%$) is innervated by post-ganglionic fibres which originate from cells in the pelvic plexus. Since the results of the present experiments were not affected by activity conducted through the smooth muscle layer, they may be more reliable than results obtained by observing

colonic contraction. A proportion of the post-ganglionic sacral parasympathetic fibres appear to end on third-order neurones in the myenteric plexus (H. Fukuda & K. Fukai, unpublished observation); the exact area innervated by these cells could not be determined by the present methods. The third fibres, however, need not be very long since the post-ganglionic fibres ascend as far as the caecum.

Responses of afferent fibres in the intramural pelvic nerves to distension of the colon and to pinching of the colonic wall were found to determine the upper limit of afferent innervation of the colon. Since the upper limit determined by the pinching method was $87{\cdot}4 \pm 6{\cdot}2\%$ ($n = 7$), which does not differ significantly ($P > 0{\cdot}5$) from the $85{\cdot}0 \pm 7{\cdot}6\%$ ($n = 5$) determined by the distension method, it seems that the correct upper limit may have been determined.

Roles of the intramural pelvic nerves

It is well known that a large part of the colon contracts almost simultaneously with defaecation and that most of the contraction is elicited by the recto- and ano-colonic reflex via the pontine and sacral defaecation reflex centres (Okada, Fukuda & Yamane, 1975; Okada, Yamane, Fukuda & Fukai, 1977; Fukuda, Fukai, Yamane & Okada, 1981). The present study shows that centrifugal activity of these reflexes is conveyed by the intramural pelvic nerves to the proximal colon, and brings about a strong contraction of the colon (Fig. 5).

The intramural pelvic nerves also convey centripetal activity from the proximal colon. When afferent activity is increased by distension of the colon with an injection of more than 50 ml of water, centrifugal activity of the nerve is produced reflexly (Fig. 5). The reflex activity elicits a contraction of the colon as well as the rectum. The threshold for this colo-colonic reflex, however, is higher than that for the intrinsic reflex via the myenteric plexus (Hukuhara & Miyake, 1958; Hukuhara, Nakayama & Nanba, 1961) and colo-colonic reflex via the vagal afferent fibres and the pelvic efferent fibres (Ohashi, 1969; Okada & Fukai, 1979). Therefore, it seems that the colo-colonic reflex elicited by afferent fibres in the intramural pelvic nerves may play a supporting role in the control of colonic motility.

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REFERENCES

FUKUDA, H., FUKAI, K., YAMANE, M. & OKADA, H. (1981). Pontine reticular unit responses to pelvic nerve and colonic mechanical stimulation in the dog. *Brain Research* **207**, 59–71.

HUKUHARA, T. & MIYAKE, T. (1958). The intrinsic reflex in the colon. *Japanese Journal of Physiology* **9**, 49–55.

HUKUHARA, T., NAKAYAMA, S. & NANBA, R. (1961). The role of the intrinsic mucosal reflex in the fluid transport through the denervated colonic loop. *Japanese Journal of Physiology* **11**, 71–79.

HULTÉN, L. (1969). Extrinsic nervous control of colonic motility and blood flow, an experimental study in the cat. *Acta physiologica scandinavica* **78**, suppl. 335, 1–116.

JENNINGS, M. A. (1938). The secretion of the colon of the cat. *Quarterly Journal of Experimental Physiology* **23**, 207–229.

OHASHI, K. (1969). Effects of distension of the proximal or the distal colon upon the movements of the other part of the colon. *Journal of the Physiological Society of Japan* **31**, 173–177 (in Japanese).

OKADA, H. & FUKAI, K. (1979). Autonomic nerve activity during the proximal–distal colonic reflex in dogs. *Journal of the Physiological Society of Japan* **41**, 384 *p*.

OKADA, H., FUKUDA, H. & YAMANE, M. (1975). The efferent activity in the pelvic nerve during the recto-rectal reflex of the dog. *Autonomic Nervous System* **12**, 278–287 (in Japanese).

OKADA, H., YAMANE, M., FUKUDA, H. & FUKAI, K. (1977). The ano-colonic reflex and parasympathetic outflow to the colon in dogs. *Autonomic Nervous System* **14**, 267–276 (in Japanese).

SCHABADASH, A. (1930). Untersuchungen zur Methodik der Methylenblaufärbung des vegetativen Nervensystems. *Zeitschrift für Zellforschung und mikroscopischen Anatomie* **10**, 221–241.

SCHMIDT, C. A. (1933). Distribution of vagus and sacral nerves to the large intestine. *Proceedings of the Society for Experimental Biology and Medicine* **30**, 739–740.

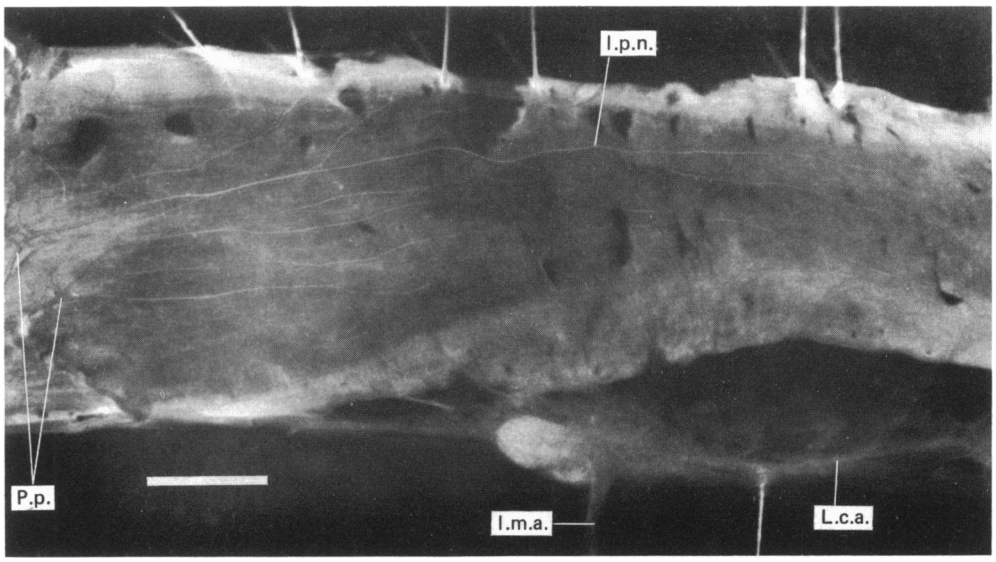
EXPLANATION OF PLATES

PLATE 1

The intramural pelvic nerves of an infant dog. The nerves (i.p.n.) were stained with Methylene Blue. P.p., pelvic plexus. I.m.a., inferior mesenteric artery. L.c.a., left colic artery. Horizontal bar indicates 10 mm.

PLATE 2

Electron micrographs of the intramural pelvic nerves. *A* and *B* show the nerves of two dogs in which the left pelvic nerve was severed and allowed to degenerate for 32 and 45 days, respectively. Horizontal bars indicate 5 μm in both *A* and *B*.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P.p.
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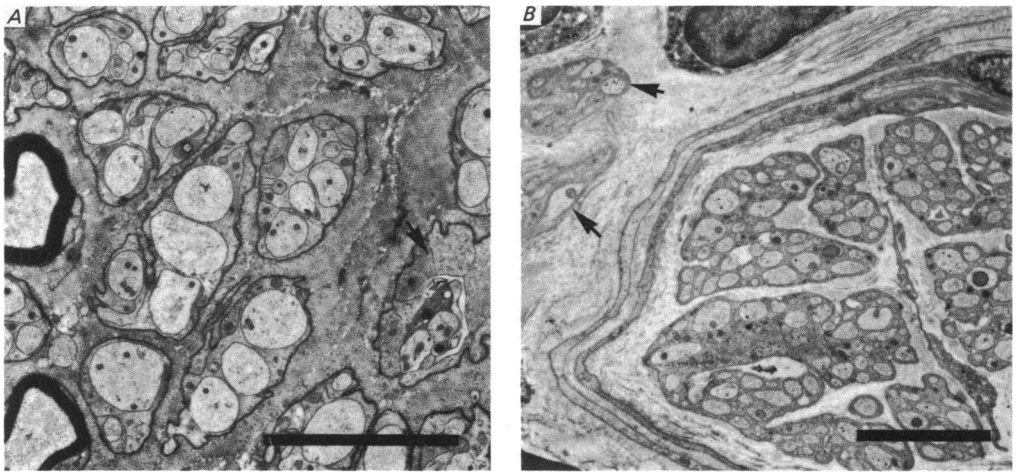
A
B