Neuropeptides in pelvic afferent pathways

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Summary. Neurochemical and pharmacological experiments have raised the possibility that several neuropeptides including, vasoactive intestinal polypeptide (VIP), peptide histidine isoleucine amide (PHI), substance P, calcitonin gene-related peptide (CGRP), neurokinin A, cholecystokinin (CCK) and opioid peptides may be transmitters in afferent pathways to the pelvic viscera. These substances are widely distributed in: 1) nerve fibers in the pelvic organs, 2) visceral afferent neurons in the lumbosacral dorsal root ganglia and 3) at sites of afferent termination in the spinal cord. Double staining immunocytochemical techniques have shown that more than one peptide can be localized in individual visceral afferent neurons and that neuronal excitatory (VIP, substance P, CCK) and inhibitory peptides (leucine enkephalin) can coexist in the same afferent cell. Studies with the neurotoxin, capsaicin, indicate that peptidergic afferent pathways are involved in the initiation of central autonomic reflexes as well as peripheral axon reflexes which modulate smooth muscle activity, facilitate transmission in automatic ganglia and trigger local inflammatory responses.

Key words. Urinary bladder; reproductive organs; large intestine; VIP; substance P; CGRP; opioid peptides; capsaicin; peptide coexistence; visceral reflexes; spinal cord.

Introduction

Afferent pathways to the pelvic viscera play an essential role in the neural regulation of various physiological functions, including micturition, defecation and reproduction[17,20]. The anatomical and electrophysiological properties of these pathways have recently been described in some detail[16,52,68,90,92,96]. On the other hand, the mechanisms underlying transmission at visceral afferent synapses are still relatively unexplored.

Presently, the neuropeptides are attracting considerable attention as putative transmitters in visceral afferent pathways. These substances are widely distributed in nerve fibers in the pelvic organs[2,3,29,30,74,77] and at sites of visceral afferent termination in the lumbosacral spinal cord[16,21,40,56]. Immunocytochemical and axonal tracing expeiments have shown that a large percentage of pelvic visceral afferent neurons contain peptides[16,18,48,63,113,114] and that more than one peptide can occur in these cells[16,25,58]. Insight into the physiological role of the neuropeptides has also been obtained by examining the effects of capsaicin on the activity of the pelvic organs[41,46,77-79,81]. Capsaicin is a very useful experimental tool for these studies since it is thought to act selectively on peptidergic afferents to release transmitters and in large doses to cause neuronal degeneration[9,27].

This chapter will review the neurochemical and pharmacological data suggesting that peptides function as transmitters in the afferent innervation of the pelvic viscera.

Peptidergic afferent innervation of the pelvic organs

The afferent innervation of the pelvic viscera (i.e., the urinary tract, distal bowel and reproductive organs) originates in the lumbosacral dorsal root ganglia and is carried by several peripheral nerves (fig. 1)[17,20,52,68]. The hypogastric and lumbar colonic nerves contain axons from the lumbar dorsal root ganglia[16,17,52,92,93], whereas the pelvic and pudendal nerves carry afferent pathways from the sacral dorsal root ganglia[16,17,52,90,102]. The pudendal nerves also contain somatic afferent axons innervating superficial and deep structures in the perineum (fig. 1), many of which are closely linked functionally with the pelvic viscera[16,18,122].

Various peptides including substance P and related tachykinins (neurokinin A, neuropeptide K and eledoisin-like peptide)[3,13,14,18,28,36,37,39,41,65,77,86,113,116,118,119], calcitonin gene related peptide (CGRP)[29,31,103,114] vasoactive intestinal polypeptide (VIP)[35,36,38,39,48,61,64,65,77], peptide histidine isoleucine amide (PHI)[14,35], galanin[7a], cholecystokinin (CCK)[14], somatostatin[14], enkephalins[14,18,21,77], and dynorphins[14] are present in nerves and ganglia within and projecting to the urogenital tract and the large intestine. The origin of these peptidergic nerves has been examined using surgical and/or chemical denervation techniques.

In rats and guinea pigs the administration of capsaicin, a neurotoxin, which acts selectively on small diameter afferent fibers to deplete transmitter stores and to induce neuronal degeneration, reduces the levels of substance P[28,41,46,47,84,104,113,118,119], neurokinin A[46,47] and CGRP[29,31,35,114] at various sites in the pelvic viscera, but does not change the levels of VIP[14,35,100,119] or enkephalins[14,35]. Surgical interruption of the extrinsic nerves (pelvic and hypogastric nerves) or removal of the lumbosacral dorsal root ganglia produces a similar selective decrease in substance P and CGRP immunoreactivity[14,29,86,87,114], whereas removal of the peripheral autonomic ganglia (pelvic ganglia) or transection of postganglionic nerves produces a genera-

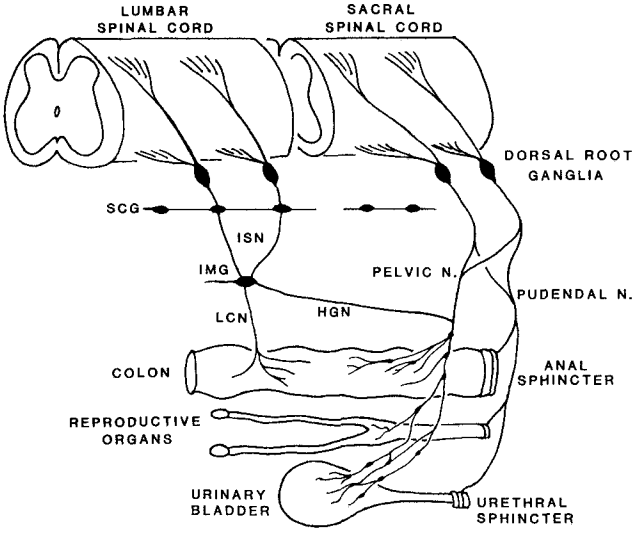

Figure 1. Diagram showing the peripheral pathways for afferent innervation of the pelvic viscera in the cat. Neurons in the sacral dorsal root ganglia send axons into the pelvic and pudendal nerves. The pelvic nerves innervate exclusively the viscera whereas the pudendal nerves innervate visceral as well as somatic structures such as the anal and urethral sphincters and the perineum. Neurons in the lumbar dorsal root ganglia send axons into the lumbar colonic (LCN) and hypogastric nerves (HGN). The latter axons pass through the sympathetic chain ganglia (SCG) and then the inferior splanchnic nerves (ISN) to the inferior mesenteric ganglia (IMG). The HGN passes caudally to join the pelvic nerve.

lized decrease in peptide immunoreactivity in certain pelvic organs as well as a decrease in markers for cholinergic and adrenergic nerves[87,114]. CGRP, substance P and VIP are not altered following the administration of 6-hydroxydopamine in doses which destroys adrenergic efferent axons[29,87,114,119]. These data indicate that CGRP, substance P and related tachykinins are present in afferent pathways to the pelvic viscera.

It should be noted, however, that the failure of capsaicin and deafferentation to change the levels of other peptides does not exclude the possibility that these peptides are also present in afferent axons. For example, VIP is contained in a large population of efferent axons in the pelvic viscera[38,73,74,77,87]. This population of VIP axons would remain after deafferentation and might obscure the changes in a smaller population of VIP afferent axons[35]. This seems to be a likely possibility since VIP has been detected in visceral afferent neurons in the dorsal root ganglia (see below)[56,59]. In addition, certain peptidergic afferents may not be sensitive to capsaicin[126].

Immunocytochemical studies[3,29,31,45] have demonstrated a widespread distribution of substance P and CGRP-containing afferent axons in the pelvic viscera. Although the tissue concentrations of CGRP are greater than those of substance P, the distribution of the two peptides is very similar. Indeed, double staining techniques in which antisera to substance P and CGRP were applied to the same tissue sections revealed that the two peptides are co-localized in axonal varicosities in various organs[31], including the intestine and the urogenital tract.

In the rat and guinea pig the highest concentrations of CGRP occur in the ureter and bladder base with lower concentrations in the bladder dome, urethra and reproductive organs including the cervix, vagina, uterus, fallopian tubes and the ovaries[29,114]. In the urogenital tract the substance P and CGRP axons are located throughout the submucosal plexus, adjacent to the epithelium, in the smooth muscle layers and around blood vessels[29,31,77,87]. The high density of these axons in proximity to the epithelial layers of the urogenital organs contrasts with the more prominent distribution of efferent cholinergic, adrenergic and VIPergic axons in the smooth muscle areas in these organs[3,87,127].

In the wall of the intestine substance P/CGRP afferent axons are distinguished from axons of intrinsic peptidergic neurons by their sensitivity to capsaicin[28,31]. Using this technique it has been shown in the guinea pig intestine, that substance P CGRP afferent fibers are relatively sparse in the myenteric plexus, but as in the urogenital organs are common beneath the epithelium and in the submucosal plexus where they follow blood vessels and form loose plexuses within the submucosal ganglia[31]. Axons which are resistant to capsaicin treatment have a different distribution.

Substance P afferent axons and varicosities are also present in prevertebral sympathetic and pelvic ganglia which provide input to the pelvic viscera[13,14,77,86]. In the inferior mesenteric ganglion (IMG, see fig. 1) of the guinea pig, the substance P varicosities form perineuronal networks around the principal ganglion cells[14,86]. At the ultrastructural level some of these varicosities were seen to make synaptic contacts with dendrites[86]. The substance P varicosities were eliminated by capsaicin pretreatment or by surgical deafferentation which interrupted the connections between the IMG and the lumbar dorsal root ganglia[14,86]. Axonal tracing studies have confirmed the afferent projections from the lumbar dorsal root ganglia to the IMG[1].

Other peptides including VIP, CCK, bombesin and dynorphin are also present in afferent pathways to the IMG, however, these substances are located primarily in axonal pathways arising from neurons in the intestine[14]. Thus, transection of the inferior splanchnic nerves central to the ganglion (fig. 1) does not alter these peptidergic axons whereas transection of the lumbar colonic nerves and hypogastric nerves peripheral to the ganglion markedly reduces their density.

The distribution of visceral afferent axons in peripheral ganglia and at various sites within the pelvic organs suggests that afferent pathways acting through the release of neurotransmitters in the periphery may have a variety of modulatory functions on nerve, smooth muscle and secretory cells in addition to the traditional sensory functions of afferent pathways. This peripheral modulatory function will be discussed in a later section.

Identification of neuropeptides in visceral dorsal root ganglion cells

Immunocytochemical techniques have been used in combination with axonal tracing methods to examine the distribution of neuropeptides in afferent neurons innervating the pelvic viscera of cat[16,21,23,25,56,58,60,63,71], rat [35,48,113,114,119] and guinea pig[13]. In the cat, fluorescent dyes were applied to the pelvic[16,63] and hypogastric nerves[23,25,58] to label various populations of visceral afferent cells. The dyes were also used in the cat and rat to label cells innervating individual organs such as the urinary bladder[25,35,60,114], colon [25,60], kidney[71,114], ureter[114] and female reproductive tract[48,119] and to label visceral and somatic afferent pathways in the pudendal nerve[63]. Colchicine was administered in many studies to increase the levels of peptides in the ganglion cells.

As shown in table 1, a large percentage of afferent neurons projecting to the pelvic and hypogastric nerves of the cat exhibited peptide immunoreactivity. Indeed, the sum of the percentages of neurons containing individual peptides exceeded 100%. The significance of this finding will be discussed below. VIP was the most common peptide occurring in 42–45% of lumbosacral visceral afferent neurons (fig. 2C). Leucine enkephalin, cholecystokinin (CCK) and substance P were present in 21–37% of the neurons, and methionine enkephalin was present in 10% of the neurons. Somatostatin was detected in very few cells (0–2%) whereas dynorphin 1–8, dynorphin 1–17 and dynorphin B were undetectable in dorsal root ganglion cells with immunocytochemical techniques[7,23,25]. Dynorphin B was identified, however, in lumbosacral dorsal root ganglia using radioimmunoassay[7].

In the same cats in which the pelvic nerve afferents were labeled another fluorescent dye was applied to the pudendal nerve to label visceral and somatic afferent neurons innervating the perineum, reproductive organs and anourethral structures[63]. VIP, CCK and methionine enkephalin were detected less frequently in these cells as compared to pelvic nerve afferent neurons, however, other peptides occurred in similar proportions in both afferent populations. The greatest difference was seen with VIP (10% vs 42%) suggesting that this peptide may be highly localized to visceral afferents. This difference between somatic and visceral cells might be even larger since those VIP cells which project to the pudendal nerve may also innervate visceral (eg. urethral and anal mucosa) rather than somatic structures (eg. cutaneous tissue).

The degree of association between VIP and visceral afferent pathways can also be examined by analyzing the data in the reverse manner to determine what proportion of VIP cells project to the viscera. It has been estimated that 66% of the VIP cells in the cat sacral dorsal root ganglia project to the pelvic nerve and 11% to the pudendal nerve; leaving 23% of the cells unidentified. These cells presumably innervate somatic structures. It should be noted, however, that pelvic nerve afferents represent only a small fraction (less than 10%) of the neurons in the sacral dorsal root ganglia. This further underscores the very strong association at this level of the spinal cord between VIP and visceral afferent pathways. As noted for peptidergic afferents in other animals[40], pep-

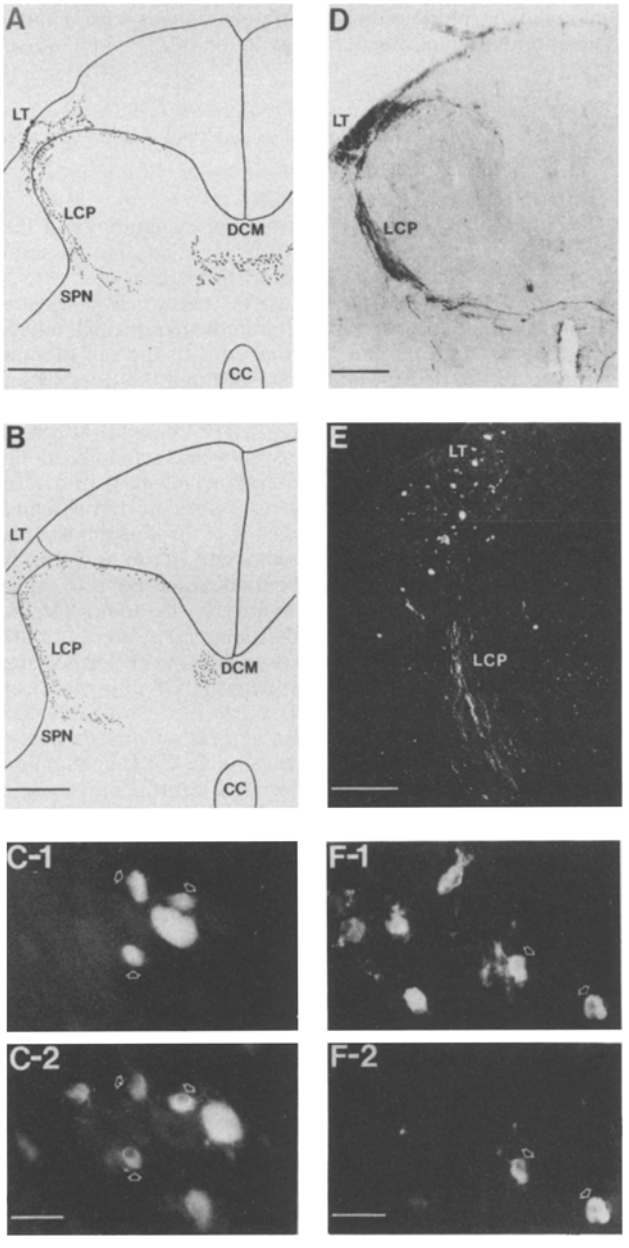

Figure 2. Afferent pathways to the sacral spinal cord. *A* Camera lucida drawing of the central projections of bladder afferents in the sacral (S_2) dorsal horn (DH) of the cat. Afferent terminals were labeled by transganglionic transport of HRP from nerves on the surface of the bladder. Labeled axons were present in Lissauer's tract (LT), the lateral collateral pathway (LCP), the sacral parasympathetic nucleus (SPN) and the dorsal commissure (DCM). *B* Afferent projections in the S_2 dorsal horn of the cat labeled by HRP injected into the external urethral sphincter. *C* Two photomicrographs of the same section through the S_2 dorsal root ganglion showing bladder afferent cells (C-1) labeled by fast blue injected into the bladder wall. Several of these labeled cells (arrows in C-1 and C-2) contain VIP-immunoreactivity (VIP-IR). Dye labeled cells were blue when visualized with UV light at 340–380 nm excitation wavelength and VIP cells were green when visualized with UV light at 430–480 nm wavelength. *D* The distribution of VIP-IR in LT and LCP of the S_2 segment of the cat spinal cord. *E* VIP-IR in the sacral dorsal horn of the S_3 segment of the human spinal cord. Large bundles of VIP axons are present in LT and smaller numbers of axons are present in lamina I on the lateral edge of the dorsal horn. *F* Co-localization of substance P-IR and VIP-IR in sacral (S_2) dorsal root ganglion cells of the cat. Substance P-IR (F-1) stained with TRITC (red color, at 530–560 nm excitation wavelength) and in F-2 the same section showing VIP-IR in two of the same ganglion cells (arrows) stained with FITC (green color at 430–480 nm excitation wavelength). VIP was detected with rabbit polyclonal antisera, whereas substance P was detected by rat monoclonal antisera. Calibration represents 250 μm in **A** and **B**, 50 μm in **C**, 300 μm in **D**, 220 μm in **E** and 60 μm in **F**. (From de Groat et al., 1986)

tide-containing cells in cat lumbosacral dorsal root ganglia were in general small to medium size cells less than 40 μm in average diameter. VIP cells had the smallest average diameter (30 μm) whereas somatostatin cells had the largest average diameter (36 μm). The size of VIP cells is consistent with the observation that VIP is present only in unmyelinated afferents[43,91] and is associated primarily with visceral afferent neurons which are on the average, considerably smaller than somatic neurons[16,63].

The distribution of peptides in afferent pathways to individual organs was studied in the cat and rat. In the cat, VIP and substance P were the most common peptides in bladder and colon afferents (table 1, range 18–25%)[23,25,60]. Substance P was also detected in 24% of renal afferents in the cat[71] and in 16% of bladder afferents in the rat[113]. Somatostatin was rarely detected in visceral afferents[25,113]. CGRP was present in a large percentage (45–90%) of the dorsal root ganglion cells innervating the kidney[114], urinary bladder[114], ureter[114] and female reproductive organs[48] in the rat. It is noteworthy that in the more rostral dorsal root ganglia (T_{10}-L_3) there was a higher percentage of these cells with CGRP than in the caudal (L_6-S_1) ganglia. This suggests that different functional groups of visceral afferents in the rat may utilize different transmitters. On the other hand, this difference was not seen in the cat where hypogastric-afferent neurons in the upper lumbar ganglia exhibited a spectrum of peptide-immunoreactivity similar to that of pelvic nerve afferent neurons in the sacral dorsal root ganglia (table 1).

Co-localization of neuropeptides in lumbosacral visceral afferent pathways

The large percentage (greater than 100%) of cat lumbosacral visceral afferent neurons containing neuropeptides suggests that some peptides must coexist in afferent neurons[63]. This was confirmed using double staining techniques where two peptides could be identified on the same tissue section (fig. 2F). Coexistence was examined in the general population of sacral afferent neurons[22] and in bladder, colon and hypogastric afferent neurons in the cat[16,25,60]. As shown in table 2 for colon afferents, coexistence of certain peptides is very common. For example, a large percentage (50–56%) of cells containing leucine enkephalin exhibit substance P or VIP; and a large percentage (51%) of VIP cells contain substance P (fig. 2F) or leucine enkephalin. However, leucine enkephalin is not co-localized with somatostatin. Similar patterns of co-localization were noted in bladder and hypogastric nerve afferents and in unlabeled afferents in the lumbosacral dorsal root ganglia.

The sum of the mean percentages of leucine enkephalin cells containing other peptides exceeds 100% indicating that more than two peptides must be contained in one cell. Preliminary studies of thin serial sections (6 μm) allowing the same DRG cell to be visualized in three consecutive sections confirmed that three peptides eg., VIP, substance P and CCK could occur in the same cell.

Co-localization of neuropeptides in unidentified dorsal root or sensory ganglion cells of the cat[75], rat[12,15,33,40,76,104,121,124] and guinea pig[30,31,69] has also been reported. Three and sometimes four peptides have been identified in single cells in the cat[75]. Among the various peptide combinations the most extreme example of co-localization is that occurring with CGRP and substance P where virtually all substance P cells contain CGRP[31,33,76]. However, a certain population of CGRP cells does not contain substance P.

Table 1. Distribution of neuropeptides in lumbosacral dorsal root ganglion cells of the cat and rat

	Cat					Rat		
	PN	HGN	PUDN	BLD	Colon	BLD	Kid-Ur.	Rep. org.
VIP	42	45	10	25	14	+	–	–
LENK	30	21	24	5	7	–	–	–
CCK	29	25	12	1	3	–	–	–
SP	24	37	21	23	18	16[b]	– +	
MENK	10	9	3	–	–	–	–	–
SS	2	0	0	2	2	0	–	–
DYN	0	0	0	0	0	–	–	–
CGRP	–	–	–	–	–	90[a]	90[a]	66–86[a]
						60[b]	90[b]	45–63[b]
Total	137	137	70	56	44			

Numbers indicate percentages of neurons exhibiting each peptide. PN, pelvic nerve; HGN, hypogastric nerve; PUDN, pudendal nerve; BLD, bladder; Kid-Ur, kidney-ureter; Rep. org., reproductive organs; VIP, vasoactive intestinal polypeptide; LENK, leucine enkephalin; CCK, cholecystokinin; SP, substance P; MENK, methionine enkephalin; SS, somatostatin; DYN, dynorphin 1–17; CGRP, calcitonin gene-related peptide. Data for HGN were obtained from upper lumbar ganglia. All other data were from sacral ganglia in the cat. (+) cells present but percentages not reported; (–), data not available. [a] T_{10}-L_3 ganglia, [b] L_6-S_1 ganglia. See text for literature references.

Table 2. Co-localization of peptides in sacral dorsal root ganglion cells innervating the colon of the cat

	LENK	SP	VIP	CCK	SS
LENK	–	56	50	6	0
SP	27	–	42	7	–
VIP	51	52	–	–	–
CCK	11	22	–	–	–
SS	0	–	–	–	–

Numbers indicate percentages of neurons in the left column exhibiting immunoreactivity for other peptides. Each percentage represents the average for several hundred cells in sacral dorsal root ganglia of two cats. (–) indicates data not available. Abbreviations are the same as in table 1. (From de Groat et al., 1987)

The significance of peptide co-localization in visceral afferent neurons is still uncertain, however, the demonstration of neuronal inhibitory (enkephalins)[18,21,23,54,55] and excitatory substances (substance P, VIP and CCK)[53,61,62,67,88,89,98,101,104,120] in the same cells raises a number of interesting possibilities. For example, enkephalins as well as excitatory peptides may be transported to central and peripheral afferent terminals and co-released (fig. 3)[24,25]. Since primary afferent terminals have opioid receptors that mediate presynaptic inhibition[54,55,72,125] enkephalins could be co-released with VIP or substance P, and then act in a negative feedback or autoinhibitory manner to depress the release of other peptides (fig. 3)[24,25]. Enkephalins might also act postsynaptically to inhibit second-order neurons. A similar action could occur at peripheral afferent terminals in the viscera (fig. 8).

It is also noteworthy that enkephalins are co-localized with various substances having synaptic excitatory actions (e.g. substance P, VIP and CCK)[53,61,62,67,88,89,101,104,120] but not with somatostatin which has synaptic inhibitory effects[104]. However, somatostatin was co-localized with substance P in nonvisceral afferent neurons[22]. Thus both inhibitory peptides coexist with excitatory peptides but not with each other.

Dynorphins and endorphins are also present in sensory ganglia[6,30,49,50,66,69,97,115,123] and in afferent projections to the spinal cord[7,23,25]. Although dynorphin immunoreactivity has not yet been detected in visceral afferent neurons, the central projections of dynorphin immunoreactive dorsal root axons to areas receiving visceral afferent input suggests that dynorphins are present in visceral afferent pathways[7,16,23,25]. Further studies will be necessary to determine whether enkephalin- and dynorphin-containing afferent systems represent the same population of dorsal root ganglion cells.

The relationship between central projections of visceral afferent pathways and the distribution of neuropeptides in the lumbosacral spinal cord.

Anatomy of visceral afferent pathways

Horseradish peroxidase (HRP) tracing experiments in the cat[19,70,90,92,93], monkey[102] and rat[94] revealed that visceral afferent pathways have a characteristic pattern of distribution in the spinal cord and that this pattern is markedly different from that of somatic afferent neurons which innervate the skin[16]. In the sacral spinal cord of the cat afferent axons from the pelvic organs pass through the dorsal root entry zone within the areas occupied by fine diameter fibers and then enter Lissauer's tract (fig. 4)[90]. Visceral afferents can extend several segments rostrally and caudally along Lissauer's tract giving off collaterals which pass in a thin shell laterally and medially around the doral horn (figs 4 and 5). At the apex and on the lateral edge of the dorsal horn this shell of visceral afferents (approximately 70 μm in width) is present in lamina I and a few fibers extend deeper into outer lamina II. Inner lamina II and laminae III-IV do not receive visceral afferent input. The bands of visceral afferents on the lateral and medial edge of the dorsal horn are termed the lateral and medial collateral pathways (LCP and MCP) of Lissauer's tract, respectively.

Axons in the LCP, which extend ventrally from Lissauer's tract through lamina I into lateral laminae V-VII and lamina X, exhibit several patterns of termination (fig. 5). In some sections, axons end in lateral lamina V (designated 'a' in fig. 5). In other sections, axons extend into medial lamina V and VII and the lower one-third of the doral gray commissure ('b' in fig. 5). Less frequently, labeled axons extend into dorsomedial lamina V ('c') or ventrally into lateral lamina VII ('d'), in the region of the sacral parasympathetic nucleus[95].

In serial transverse sections the intensity of labeling in the LCP exhibits considerable variation from section to section. Horizontal sections show that this variability is related to a

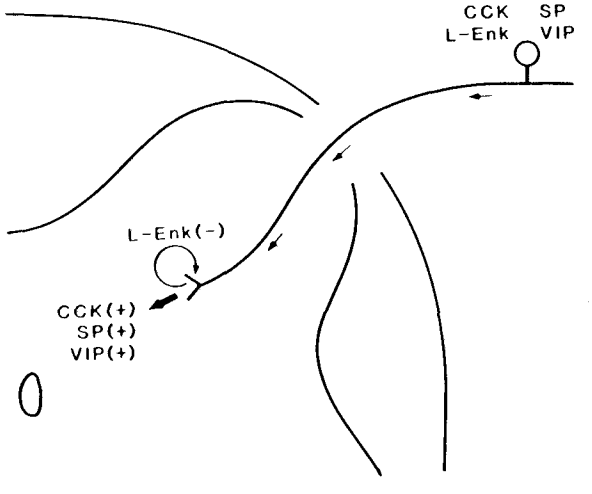

Figure 3. Diagram illustrating possible function of leucine enkephalin (L-Enk) in primary afferent neurons. L-Enk may be released at afferent terminals in the spinal cord and interact with opioid receptors on the terminals to mediate feedback inhibition of the release of excitatory transmitters, such as substance P (SP), vasoactive intestinal polypeptide (VIP) and cholecystokinin (CCK). L-Enk-IR has been identified in dorsal root ganglion cells containing either SP, VIP or CCK. However, it is not known whether all 4 peptides are localized in the same population of primary afferent neurons. (From de Groat et al., 1986)

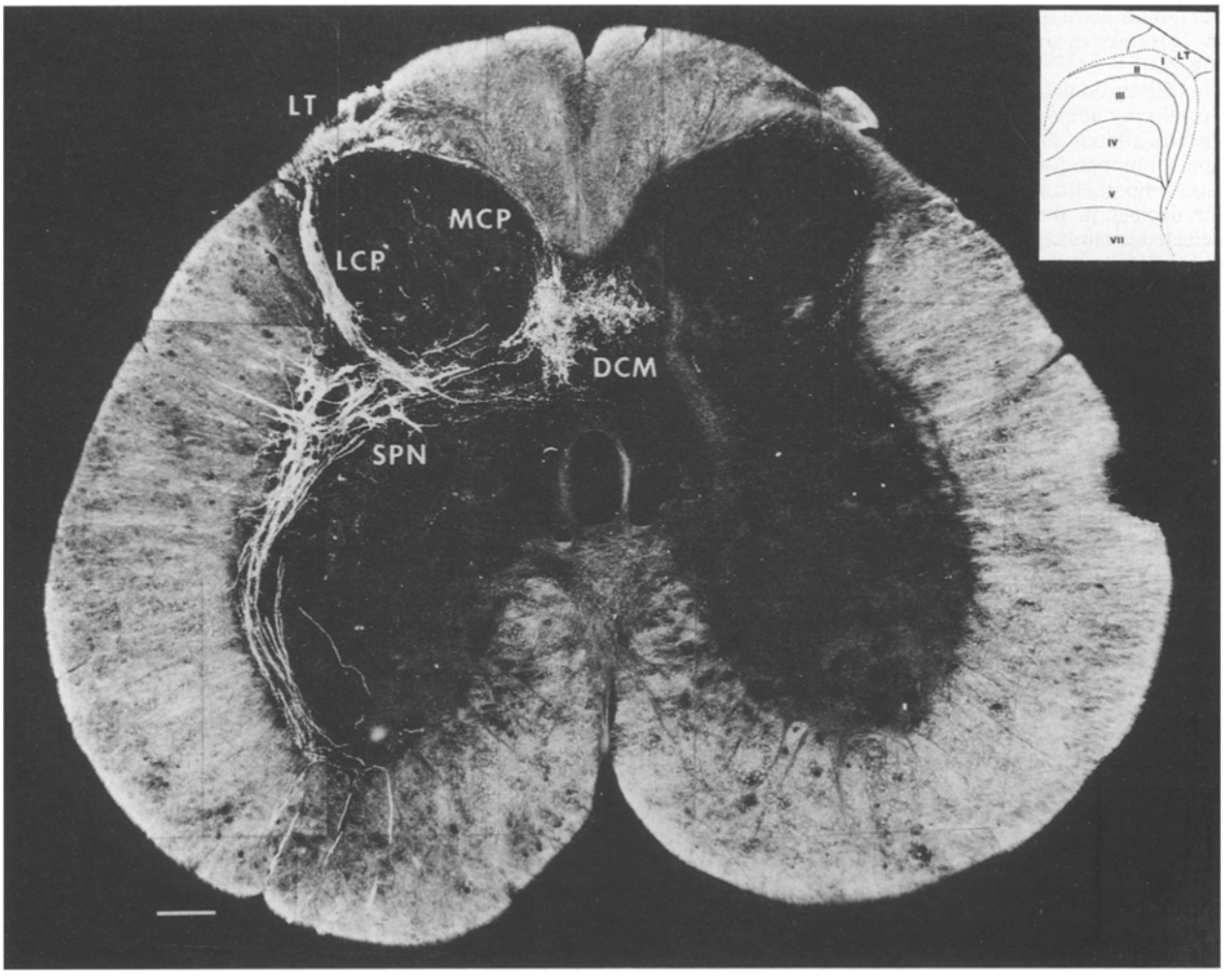

Figure 4. Transverse section of S_2 spinal cord showing labeling of primary afferents and preganglionic neurons after application of HRP to the left pelvic nerve in the cat. Pelvic afferents enter Lissauer's tract (LT). Afferent collaterals enter lamina I and extend laterally in a large bundle, the lateral collateral pathway (LCP), into the area of the sacral parasympathetic nucleus (SPN). Collaterals also extend medially in a smaller group, the medial collateral pathway (MCP), into the dorsal gray commissure (DCM), where they expand into a large terminal field ipsilaterally and contralaterally. Small numbers of afferents are also present in contralateral laminae I and V. This photomicrograph was made using darkfield illumination with polarized light. Bar represents 200 μm. Inset shows the laminar organization of the sacral dorsal horn according to Rexed. (From Morgan et al., 1981)

periodic grouping of collateral bundles along the length of the spinal cord. The average distance between bundles (center to center) is approximately 215 μm, while the average bundle width is approximately 100 μm. Axons extending from lamina I medially through lamina V and VII to the dorsal gray commissure also occur in bundles.

The MCP consists of a thin band of axons which passes dorsoventrally along the medial edge of the dorsal horn into the region of the dorsal gray commissure, where the pathway expands into diffuse terminal fields on both sides of the midline (fig. 4 and areas 'f' and 'g' in fig. 5). Some axons in the MCP extend into lamina V, to overlap with the dorsomedial projections from the LCP ('e').

A similar pattern of central afferent projections has been noted for: 1) afferent pathways to individual pelvic organs in the cat (urinary bladder, colon, uterus) (fig. 2A)[16,23], 2) pudendal afferent input from deep perineal structures such as the urethra and external urethral sphincter (fig. 2B)[16,102,117], 3) the hypogastric and lumbar colonic nerve pathways in the cat[92,93] and 4) pelvic nerve afferents in the monkey and rat[94,96]. One spinal projection noted only in the rat consisted of a longitudinal bundle of pelvic nerve axons ventral to the central canal[94].

Distribution of neuropeptides at sites of visceral afferent termination in the spinal cord

Sites in the lumbosacral spinal cord which receive visceral afferent input also receive dense peptidergic projections. As might be expected those peptides present in dorsal root ganglion cells (eg., VIP, substance P, CGRP, CCK and opioid peptides) are also very prominent in the spinal dorsal horn. However, there are significant differences in the spinal distribution of these peptides.

For example, VIP exhibits an unusual segmental distribution which suggests an association with visceral afferents[4-6,16,21,23,34,43,56,57,59,109,111,117]. In various species VIP-IR is considerably higher in the sacral segments than in other segments of the spinal cord. This is most striking in the cat[6,16,21,34,35,43,56,59] and man[4,5,23,57] but was also noted in monkeys and guinea pigs[16,23,34]. PHI which is co-localized with VIP in neurons has a similar distribution in the human, rat and cat spinal cord[5,10,35].

In the lumbosacral spinal cord the VIP-containing axons also exhibit a striking similarity to certain components of the visceral afferent pathways (fig. 2D,E)[90,94,96]. In the cat, which has been studied in the most detail VIP-immunoreactivity in

the lumbosacral spinal cord is distributed most prominently in Lissauer's tract and in the area of the LCP (fig. 2D)[6,21,35,43,56,59]. VIP axons extend ventromedially from the LCP through laminae V-VII into the ventral region of the dorsal gray commissure (lamina X) (fig. 6A), similar to the visceral afferent projections to area 'b' illustrated in figure 5. Small numbers of VIP axons also extend into the sacral parasympathetic nucleus and in some cats to pudendal motor nucleus in the ventral horn. Very few VIP fibers are detected in laminae II-IV of the dorsal horn. A similar distribution was noted in man[4,23,57] (fig. 2E), monkey[23,34] and rodents[34,35,109]. Electron microscopy has revealed that VIP-immunoreactivity (VIP-IR) in cat primary afferent pathways is localized in unmyelinated axons which form large (1–5 μm) varicosities in the LCP[43,91].

In the LCP, VIP-IR exhibits a distinctive periodic pattern in the rostrocaudal axis, consisting of bundles of axons spaced at approximately 210 μm along the length of the cord (fig. 7)[6,43,56,59]. This pattern is similar to the periodic distribution of visceral afferent axons in this region[90]. VIP-IR in Lissauer's tract and the LCP of the cat is almost completely eliminated by lumbosacral dorsal rhizotomy (fig. 6) although VIP-IR at other sites (lamina X) remains. The latter may be associated with VIP neurons intrinsic to the spinal cord[34,109]. VIP-IR in the spinal cord of the cat is also significantly decreased by transection of the pelvic nerve[35], whereas electrical stimulation of the pelvic nerve releases VIP-IR from the spinal cord[8]. These observations provide considerable indirect support for a transmitter function of VIP in sacral visceral afferents.

Substance P-IR also coincides with sites of visceral afferent termination in the lumbosacral spinal cord[43,59], however, in contrast to VIP-IR, substance P-IR is distributed more uniformly at all segmental levels[16,32,40,43,59,104,112]. Substance P-IR and VIP-IR also exhibit certain differences in the sacral spinal cord of the cat (fig. 6). For example, substance P is distributed more evenly on the medial and lateral sides of the dorsal horn and in deeper lamina (II and III). In addition substance P axons from the LCP pass into the dorsal part rather than the ventral part of the dorsal gray commissure (fig. 6). Substance P-IR is reduced to lesser extent than VIP-IR by sacral dorsal rhizotomy and only certain areas such as the ventral LCP are clearly affected (fig. 6)[59]. Substance P axons in this area exhibit a periodic distribution similar to VIP axons[16,21,59]. Substance P-IR and VIP-IR are both present in the lumbosacral autonomic nuclei. Substance P-IR has also been identified in the human sacral spinal cord in the lateral marginal zone of the dorsal horn and in the area of the sacral autonomic nucleus[23,26].

Thus substance P inputs to the spinal dorsal horn and intermediate gray matter are clearly, more complicated than VIP inputs and are likely to be derived from multiple sources including somatic and visceral afferent pathways as well as neurons within the central nervous system[26,40,104]. However, certain components of the substance P afferent pathways correspond to visceral afferent terminations in the cord.

CGRP[33] and CCK[16,21,40,43] are distributed across all spinal segmental levels in the cat and like substance P are most prominent in the superficial laminae of the dorsal horn. Projections into lamina V and X also occur. The concentrations in the spinal cord are reduced by dorsal rhizotomy[21,33]. Dynorphin B[7] and dynorphin 1–17[23] also exhibit a prominent localization in Lissauer's tract and in the marginal zone (LCP) on the lateral edge of the dorsal horn in the sacral spinal cord of the cat. The dynorphin axons exhibit a periodic distribution in the LCP similar to VIP and are markedly reduced in density following sacral dorsal rhizotomy[7,23]. On the other hand, leucine and methionine enkephalin-immunoreactivity in the LCP is not changed following rhizotomy.

Somatostatin distribution in the sacral dorsal horn of the cat is markedly different from other peptides[43]. Somatostatin containing varicosities are present in lamina II-III and in the region of the sacral parasympathetic nucleus, but are absent in the LCP. This distribution is consistent with the results of the studies on dorsal root ganglia indicating that visceral afferents rarely contain somatostatin[16,23,63,113].

Peptide distributions in the rat lumbosacral spinal cord[10a,12,15,32–35,40,104,109,110,112,126] in general follow the pattern seen in the cat but there are some differences. For example, the high level of VIP in the sacral segments as compared to other cord segments is not as striking as in the cat[34], suggesting that VIP may have widespread functions in the rat. Another difference which is particularly relevant to visceral afferent pathways is the distribution of peptides in the area around the central canal. Longitudinal bundles of peptidergic axons are present dorsal and ventral to the central canal. Afferent pathways to the pelvic viscera project into the ventral bundle[94]. The density of CGRP[33,35], substance P[15,35,51,109], VIP/PHI[35,51], and galanin[10a] immunoreactivity in this region as well as in the superficial lamina of the dorsal horn[51] is markedly reduced by transection of the dorsal roots or the administration of capsaicin neonatally. This indicates that peptidergic afferents make an important contribution to the ventral bundle. CCK immunoreactivity[51,55a,109] has also been identified in these areas; however, there are concerns about the interpretation of CCK immunocytochemistry. For example, capsaicin treatment reduces the numbers of CCK positive axons identified in the spinal dorsal by immunocytochemistry but does not change the CCK levels determined by radioimmunoassay[110]. Recently it has been reported that many antibodies used for the identification of CCK in the rat cross-react with CGRP. This raises the possibility that CCK-like immunoreactivity in primary afferent neurons in the rat may represent CGRP or a similar peptide.

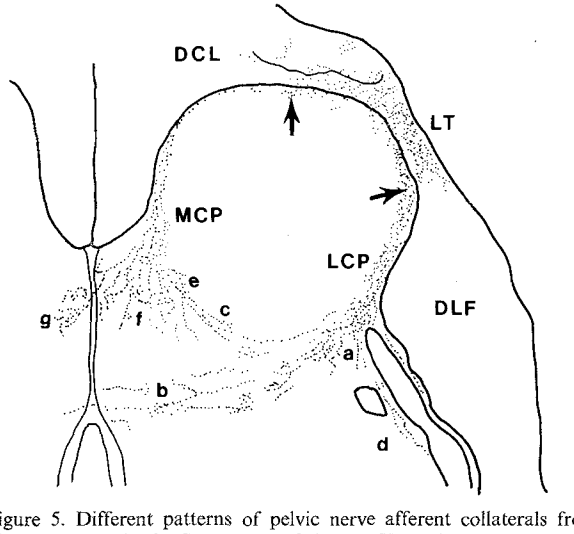

Figure 5. Different patterns of pelvic nerve afferent collaterals from Lissauer's tract in the S_2 segment of the cat. Ventral roots were cut to eliminate efferent labeling. Camera lucida drawings are composed from seven individual sections. These patterns were observed consistently in all experiments in various combinations. The lateral collateral pathway (LCP) exhibited four patterns (a, b, c, d). Many axons ended at the junction of laminae I and V (a), while others continued into lamina V to end in the lower third of the dorsal gray commissure (b). Less frequently, LCP axons ended dorsomedially in medial lamina V (c) or extended into lateral lamina VII (d). The medial collateral pathway (MCP) exhibited three patterns (e, f, g). The most common were the ipsilateral (f) and the contralateral (g) terminal fields in the upper two-thirds of the dorsal gray commissure. Less frequently, axons extended laterally into medial lamina V (e). Arrows show boundaries of Lissauer's tract. DLF, dorsolateral funiculus; DCL, dorsal column; CC; central canal. (From Morgan et al., 1981)

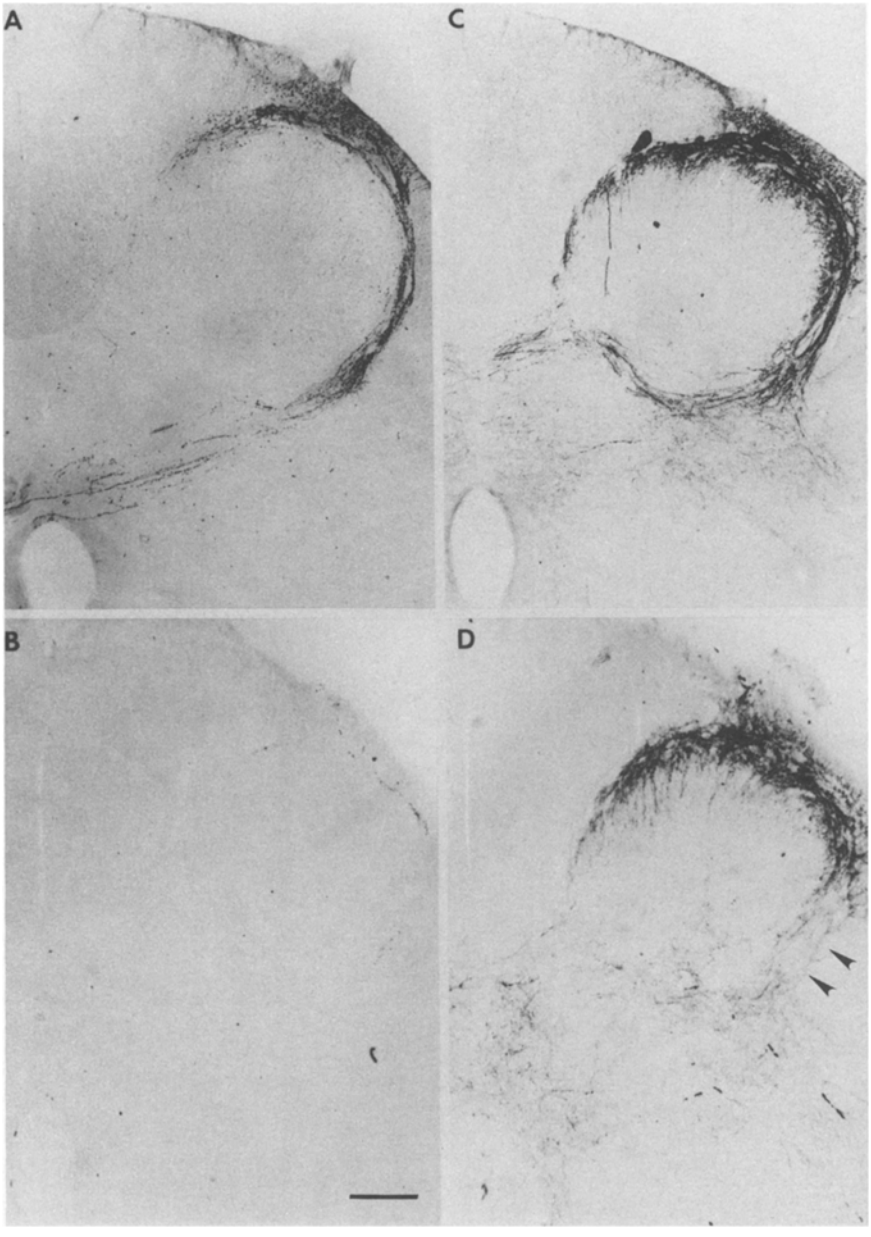

Figure 6. Transverse sections of the S_2 dorsal horn showing VIP-IR (A, B) and substance P-IR (C, D) in normal (A, C) and 5 weeks after unilateral sacral dorsal and ventral root transection (B, D). Note: VIP-IR in the lateral collateral pathway passed ventrally to the region of the central canal (A), whereas substance P-IR passed dorsally into the dorsal gray commissure (C). Deafferentation caused a marked reduction in VIP-IR in Lissauer's tract and lamina I (B) and only a slight reduction in substance P-IR on the medial side of the dorsal horn and in lateral lamina I (D, arrows). Calibration bar = 200 µm. (From Kawatani et al., 1985)

Peptidergic (CGRP, substance P, VIP, CCK, galanin) axons are also very prominent in the lumbosacral autonomic nuclei in the rat[10a,33,34,109,110]. These axons parallel the distribution of visceral afferent projections[94] and are reduced in number by dorsal rhizotomy or capsaicin treatment[33–35,40,51,104].

Physiological role of peptidergic visceral afferent neurons

It is now recognized that afferent neurons can release neuropeptides from peripheral terminals in the viscera as well as from central terminals in the spinal cord and brain[8,45–47,54,78,79,81,126]. Thus, visceral dorsal root ganglion cells are likely to have multiple functions including: 1) transmission of sensory and reflexogenic signals to the central nervous system and 2) modulation of various peripheral mechanisms such as effector organ activity, local blood flow, autonomic ganglionic transmission, and afferent receptor sensitivity (fig. 8)[40,47,78,79]. It seems appropriate, therefore, to consider the visceral dorsal root ganglion cell and its processes as an anatomical substrate for mediating peripheral efferent as well as afferent responses (fig. 8). Several examples of efferent-afferent functions of peptidergic neurons have been demonstrated in the pelvic viscera using the neurotoxin, capsaicin[9,42,45–47,80–85,78,105–107,120]. These experiments have been conducted primarily on the neural pathways to the urinary tract of the rat and guinea pig; which are very sensitive to capsaicin.

Several laboratories have examined the effect of capsaicin on the micturition reflex[42,79,83–85,113]. Micturition is triggered by pelvic nerve afferent pathways which convey activity from

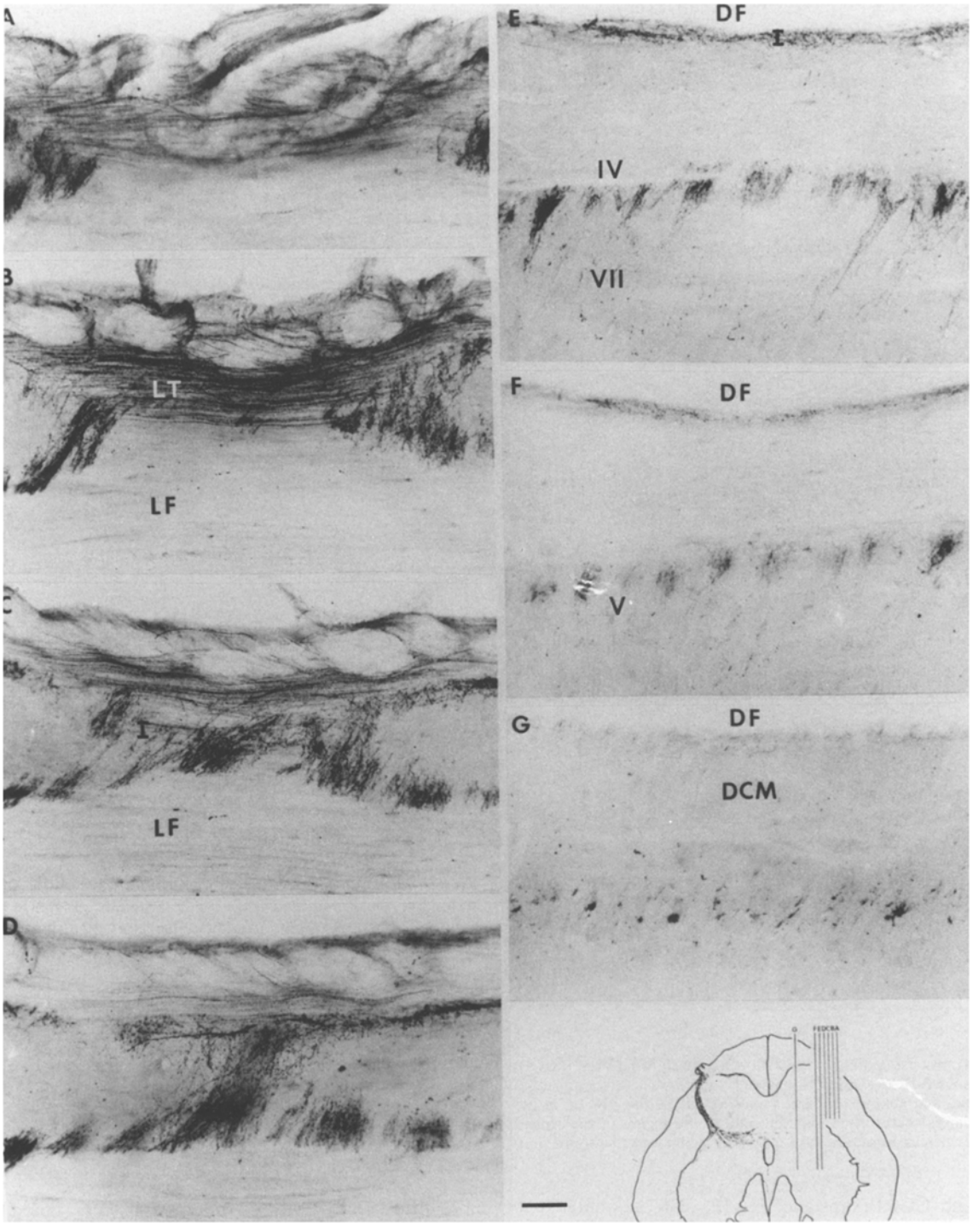

Figure 7. Sagittal sections showing the VIP distribution in the S_1 segment of the spinal cord. Dorsal edge is at the top of each photomicrograph and rostral is to the left. *A* VIP-IR in dorsal rootlets, Lissauer's tract, and lamina I. *B* Rostrocaudal bundles of VIP axons in Lissauer's tract (LT) and lamina I. Dorsoventral VIP fibers in lamina I. *C* Dorsoventral VIP fibers start to form discrete bundles. Some VIP axons pass dorsoventrally in lamina I (I) from rostrocaudal bundles in Lissauer's tract. *D* Discrete bundles of VIP axons in the ventral part of lateral lamina I. VIP-IR is not present in inner lamina II and laminae III-IV (IV). *E, F* Periodic appearance of VIP-IR at the junction between lateral laminae I and V. Ventral projections of VIP axons end abruptly in ventral lamina V (V). A few VIP fibers extend into lamina VII (bottom) and are present in the medial collateral pathway in lamina I (top of photomicrograph). *G* VIP-IR in lateral collateral pathway breaks up into individual fibers or small bundles with a lateromedial orientation (bottom of the photomicrograph); VIP-IR on the medial side of the dorsal horn (medial collateral pathway, top of photomicrograph). DCM, dorsal commissure; DF, dorsal funiculus; LF, lateral funiculus. Calibration bar = 200 μm. (From Kawatani et al., 1985)

tension receptors in the bladder wall[17,18,20]. These afferents trigger reflex firing in the sacral efferent pathways to produce detrusor contractions and bladder emptying. Administration of capsaicin markedly alters the micturition reflex. The acute effects of capsaicin administered systematically (50 mg/kg, s.c.) or topically to the bladder are to enhance bladder activity and to facilitate micturition[79-81,83-85]. For example, topical application of capsaicin to the bladder elicits an initial tonic contraction followed by large rhythmic contractions. Various pharmacological and neurophysiological data indicate that the tonic contraction is due to release of substance P or a related tachykinin from afferent fibers in the bladder. These substances exert direct excitatory actions on the bladder smooth muscle[83-85,105]. The rhythmic contractions are attributed to tachykinin-induced facilitation of the micturition reflex pathway possibility due to stimulation or sensitization of tension receptors in the bladder wall. These effects of capsaicin are mimicked by the topical administration of exogenous substance P to the bladder[79,85]. The effects are eliminated either by chronic deafferentation, by pretreatment with capsaicin or by the administration of a substance P antagonist[79,83,105].

The systemic administration of capsaicin to urethane anesthetized adult rats also produces a delayed-onset (> 30 min) depression of the micturition reflex[42]. The duration of this depression has not been reported, however, it is likely to be prolonged since pretreatment of adult rats with capsaicin (50 mg/kg, s.c.) four days prior to study significantly alters the bladder volume and intravesical pressure necessary to induce micturition in urethane anesthetized animals[83]. The intrathecal or intracerebroventricular administration of capsaicin to adult rats elicits a similar prolonged (4–60 days) depression of the micturition reflex, suggesting that the depletion of peptidergic afferent projections in the central nervous system is in part responsible for the effects of capsaicin. Furthermore, since capsaicin pretreatment did not alter the magnitude of the bladder contractions during micturition or the responses of the bladder to stimulation of somatic afferent pathways, it was concluded that capsaicin does not act on the peripheral efferent pathway or the central reflex mechanism involved in the micturition reflex but that it interferes with the afferent pathways from the bladder which relay pressure-volume information to the central nervous system[83,106].

The administration of capsaicin to neonatal rats produces a greater deficit in micturition[42,79,105,106,113,114], consistent with the more prominent effect of the neurotoxin on peptidergic afferent fibers in neonatal animals. Animals 2–6 months of age treated with capsaicin as neonates exhibited a 50% loss of bladder afferent innervation[113], an almost complete loss of substance P afferent innervation[113] and a marked reduction of substance P and CGRP levels in the bladder[113,114]. These animals had hypertrophied bladders (4-fold increase in weight of the empty bladders), which contained large volumes of urine (greater than 5 ml volume versus less than 1 ml for untreated animals). There was also an increased incidence of bladder calculi and enlargment of the prostate gland. In addition, the bladders from capsaicin-treated animals did not exhibit excitatory reflexes in response to distension. However, strips of bladder smooth muscle from these animals did respond normally to exogenous acetylcholine, substance P and electrical field stimulation, indicating that the efferent excitatory pathway to the bladder was intact[105].

The neonatally capsaicin-treated animals also formed less urine in response to a water load, suggesting that they had impaired renal function[42]. This was not observed in animals treated with capsaicin after the early postnatal period.

Capsaicin administered to neonatal and adult rats and guinea pigs also affects the afferent innervation of the ureter[11,29,44-47,78,82,107,114]. This was demonstrated immunocytochemically as a depletion of CGRP[29,114], substance P and related tachykinins[45,78,107] in the ureter and at the ultrastructural level as a degeneration of a large percentage of the unmyelinated axons in the ureter[11,44].

The functional consequences of capsaicin-induced destruction of ureter-afferents is uncertain; however, pharmacological studies indicate that peptides released from afferents might have multiple functions in the ureter[46,47,78,82,107]. For example, electrical or chemical stimulation of the innervation of the ureter elicits: 1) inhibition of ureteral motility, 2) facilitation of ureteral motility and 3) an inflammatory response characterized by plasma protein extravasation. These effects are mimicked by acute administration of capsaicin and can be blocked by pretreatment with large doses of capsaicin. It was concluded that the responses are mediated by substances released from afferent nerves.

Various evidence indicates that tachykinins and CGRP are mediators of the afferent evoked responses in the ureter since capsaicin is known to release these substances in various tissues[78]. For example, exogenous substance P and neurokinin A mimic the facilitatory effect of capsaicin on ureteral motility[47,78,82], whereas CGRP mimics the inhibitory effect[47]. In addition substance P antagonists block the facilitatory response induced by capsaicin[46].

Capsaicin pretreatment also influences spontaneous motility of the rat ureter[82]. Isolated segments of rat ureters maintained in vitro are normally quiescent. However, 50% of ureteral segments obtained from rats pretreated with capsaicin exhibit spontaneous rhythmic activity. This suggests that capsaicin sensitive nerves may have an inhibitory role in controlling ureteral motility.

Peptidergic afferent pathways may also control pelvic visceral function by influencing transmission in autonomic ganglia. As noted in a preceding section, peptidergic afferent varicosities are prominent in autonomic ganglia[13,14,77,86] and afferent fibers are located in close proxomity to the ganglion cells[1,14,86]. Furthermore, many peptides which are contained in visceral afferent neurons (e.g. substance P, CGRP, VIP and CCK) have facilitatory and/or excitatory effects when administered exogenously to ganglion cells[61,62,67,88,89,108,120]. In the lumbosacral visceral pathways the most detailed analysis of visceral afferent inputs to autonomic ganglion cells has been obtained in the inferior mesenteric ganglion of the guinea pig[13,14,67,120]. It has been shown that electrical stimu-

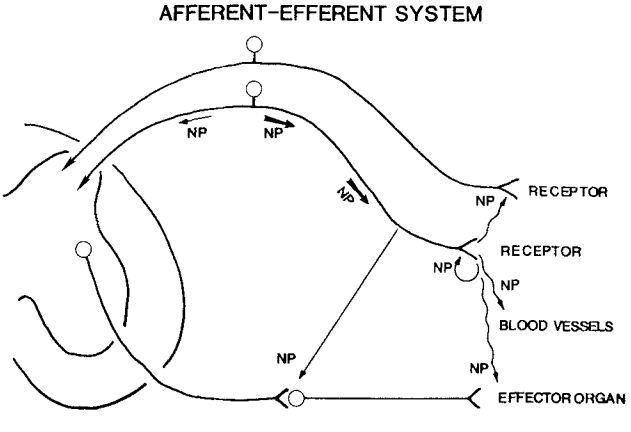

Figure 8. Diagram showing the possible functions of neuropeptides (NP) in visceral afferent neurons. Neuropeptides transported to central afferent terminals may function as transmitters or modulators at synapses in the spinal cord. Neuropeptides transported peripherally may regulate: 1) transmission in autonomic ganglia, 2) effector organ activity, 3) afferent receptor sensitivity, and 4) local blood flow. Thus visceral dorsal root ganglion cells and their processes appear to represent an anatomical substrate for the mediation of efferent as well as afferent responses.

lation of afferent axons from the lumbar dorsal root ganglia elicits a noncholinergic slow EPSP in the ganglion cells[67,120]. This response could be mimicked by the application of substance P to the ganglion cells and could be blocked by the administration of a substance P antagonist or by prolonged treatment with capsaicin, which depletes substance P levels in ganglia.

Other peptides (VIP, CCK, neurokinin A)[62,88,89,108], also produce slow noncholinergic depolarizations of autonomic ganglion cells. These substances have been implicated as transmitters in peripheral afferent pathways passing from the intestine to the inferior mesenteric ganglion[14]. However, they have not yet been shown to mediate excitatory synaptic potentials elicited by afferent input from the dorsal root ganglion cells.

Summary

In summary, pharmacological and immunocytochemical studies have raised the possibility that synaptic transmission at visceral afferent terminals in the spinal cord and in the periphery may involve various peptide transmitters and that both excitatory and inhibitory transmitters may be released from the same afferent neuron. It is also clear that while many peptides are present in afferents there are considerable differences in the numbers of neurons containing each peptide. For example, VIP and CGRP are present in a large percentage of visceral afferents whereas other peptides such as somatostatin are not present or are in very few cells. In addition, some peptides (eg, VIP) are prominently associated with visceral neurons whereas others (substance P) are equally distributed in visceral and somatic neurons. These findings raise the possibility that there may be a preferential distribution of some peptides between visceral and somatic systems and that certain of these substances might be useful markers of specific populations of afferents.

On the other hand, it is clear that peptides are not likely to be organ specific since there is considerable overlap in the spectrum of peptidergic afferents innervating the urinary bladder and colon of the cat. This finding is of particular interest since the reflex mechanisms in these two organs are controlled by different types of afferent fibers: Aδ afferents which initiate micturition and C-fiber afferents which stimulate defecation[20,23]. Thus, different functional groups of afferents innervating the pelvic viscera may utilize the same peptide transmitters.

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1 Aldskogius, H., Elfvin, L.-G., Forsman, C. A., Primary sensory afferents in the inferior mesenteric ganglion and related nerves of the guinea pig: An experimental study with anterogradely transported wheat germ agglutinin-horseradish peroxidase conjugate. J. auton. nerv. Syst. *15* (1986) 179–190.

2 Alm, P., Alumets, J., Hakanson, R., and Sundler, F., Peptidergic (vasoactive intestinal peptide) nerves in the genito-urinary tract. Neuroscience *2* (1977) 751–757.

3 Alm, P., Alumets, J., Brodin, E., Hakanson, R., Nilsson, G., and Sjoberg, N. O., Peptidergic (substance P) nerves in the genito-urinary tract. Neuroscience *3* (1978) 419–425.

4 Anand, P., Gibson, S. J., McGregor, G. P., Blank, M. A., Ghatei, M. A., Bacarese-Hamilton, A. J., Polak, J. M., and Bloom, S. R., A VIP-containing system concentrated in the lumbosacral region of human spinal cord. Nature *305* (1983) 143–145.

5 Anand, P., Gibson, S. J., Yiangou, Y., Christofides, N. D., Polak, J. M., and Bloom, S. R., PHI-like immunoreactivity co-locates with the VIP-containing system in human lumbosacral spinal cord. Neurosci. Lett. *46* (1984) 191–196.

6 Basbaum, A. I., and Glazer, E. J., Immunoreactive vasoactive intestinal polypeptide is concentrated in the sacral spinal cord: A possible marker for pelvic visceral afferent fibers. Somatosensory Res. *1* (1983) 69–82.

7 Basbaum, A. I., Crus, L., and Weber, E., Immunoreactive dynorphin B in sacral primary afferent fibers of the cat. J. Neurosci. *6* (1986) 127–133.

7a Bauer, F. E., Christofides, N. D., Hacker, G. W., Blank, M. A., Polak, J. M., and Bloom, S. R., Distribution of galanin immunoreactivity in the genitourinary tract of man and rat. Peptides *7* (1986) 5–10.

8 Blank, M. A., Anand, P., Lumb, B. M., Morrison, J. F. B., and Bloom, S. R., Release of vasoactive intestinal polypeptide-like immunoreactivity (VIP) from cat urinary bladder and sacral spinal cord during pelvic nerve stimulation. Dig. Dis. Sci. *29* (1984) 10.

9 Buck, S. H., and Burks, T. F., The neuropharmacology of capsaicin: Review of some recent observations. Pharmacol. Rev. *38* (1986) 179–226.

10 Christofides, N. D., Polak, J. M., and Bloom, S. R., Studies on the distribution of PHI in mammals. Peptides *5* (1984) 261–266.

10a Ch'ng, J. L. C., Christofides, N. D., Anand, P., Gibson, S. J., Allen, Y. S., Su, H. C., Tatemoto, K., Morrison, J. F. B., Polak, J. M., and Bloom, S. R., Distribution of galanin immunoreactivity in the central nervous system and the responses of galanin-containing neuronal pathways to injury. Neuroscience *16* (1985) 343–354.

11 Chung, K., Schwen, R. J., and Coggeshall, R. E., Ureteral axon damage following subcutaneous administration of capsaicin in adult rats. Neurosci. Lett. *53* (1985) 221–226.

12 Dalsgaard, C. J., Vincent, S. R., Hökfelt, T., Lundberg, J. M., Dahlstrom, A., Schultzberg, M., Dockray, G. J., and Cuello, A. C., Co-existence of cholecystokinin- and substance P-like peptides in neurons of the dorsal root ganglia of the rat. Neurosci. Lett. *33* (1982) 159–163.

13 Dalsgaard, C. J., Hökfelt, T., Elfvin, L. G., Skirboll, L., and Emson, P., Substance P containing primary sensory neurons projecting to the inferior mesenteric ganglion: Evidence from combined retrograde tracing and immunohistochemistry. Neuroscience *7* (1982) 647–654.

14 Dalsgaard, C. J., Hökfelt, T., Schultzberg, M., Lundberg, J. M., Terenius, L., Dockray, G. J., and Goldstein, M., Origin of peptide-containing fibers in the inferior mesenteric ganglion of the guinea-pig: immunohistochemical studies with antisera to substance P, enkephalin, vasoactive intestinal polypeptide, cholecystokinin and bombesin. Neuroscience *9* (1983) 191–211.

15 Dalsgaard, C. J., Haegerstrand, A., Theodorsson-Norheim, E., Brodin, E., and Hökfelt, T., Neurokinin A-like immunoreactivity in rat primary sensory neurons; coexistence with substance P. Histochemistry *83* (1985) 37–39.

16 de Groat, W. C., Spinal cord projections and neuropeptides in visceral afferent neurons. Prog. Brain Res. *67* (1986) 165–187.

17 de Groat, W. C., and Booth, A. M., Autonomic systems to the urinary bladder and sexual organs, in: Peripheral Neuropathy, vol. 1, pp. 285–299. Eds P. J. Dyck, P. K. Thomas, E. H., Lambert and R. Bunge. W. B. Saunders Co., Philadelphia 1984.

18 de Groat, W. C., and Kawatani, M., Neural control of the urinary bladder: Possible relationship between peptidergic inhibitory mechanisms and detrusor instability. Neurol. Urodynam. *4* (1985) 285–300.

19 de Groat, W. C., Nadelhaft, I., Morgan, C., and Schauble, T., Horseradish peroxidase tracing of visceral efferent and primary afferent pathways in the sacral spinal cord of the cat using benzidine processing. Neurosci. Lett. *10* (1978) 103–108.

20 de Groat, W. C., Nadelhaft, I., Milne, R. J., Booth, A. M., Morgan, C., and Thor, K., Organization of the sacral parasympathetic reflex pathways to the urinary bladder and large intestine. J. auton. nerv. Syst. *3* (1981) 135–160.

21 de Groat, W. C., Kawatani, M., Hisamitsu, T., Lowe, I., Morgan, C., Roppolo, J., Booth, A. M., Nadelhaft, I., Kuo, D., and Thor, K., The role of neuropeptides in the sacral autonomic reflex pathways of the cat. J. auton. nerv. Syst. *7* (1983) 339–350.

22 de Groat, W. C., Kawatani, M., Houston, M. B., and Erdman, S. L., Co-localization of VIP, substance P, CCK, somatostatin and enkephalin immunoreactivity in lumbosacral dorsal root ganglion cells of the cat. Vth International Washington Spring Symposium, May 28–31, Washington, DC 1985, Abstr. p. 48.

23 de Groat, W. C., Kawatani, M., Hisamitsu, T., Booth, A. M., Roppolo, J. R., Thor, K., Tuttle, P., and Nagel, J., Neural control of micturition: The role of neuropeptides. J. auton. nerv. Syst., Suppl (1986) 369–387.

24 de Groat, W. C., Lowe, I., Kawatani, M., Morgan, C. W., Kuo, D., Roppolo, J. R., and Nagel, J., Identification of enkephalin immunoreactivity in sensory ganglion cells. J. auton. nerv. Syst., Suppl. (1986) 361–368.

25 de Groat, W. C., Kawatani, M., Houston, M. B., Rutigliano, M., and Erdman, S., Identification of neuropeptides in afferent pathways to the pelvic viscera of the cat, in: Organization of the Autonomic

Nervous System: Central and Peripheral Mechanisms. A.R. Liss, Inc., New York (in press).
26 de Lanerolle, N.C., and LaMotte, C.C., The human spinal cord: Substance P and methionine-enkephalin immunoreactivity. J. Neurosci. *2* (1982) 1369–1386.
27 Fitzgerald, M., Capsaicin and sensory neurones – a review. Pain *15* (1983) 109–130.
28 Furness, J.B., Papka, R.E., Della, N.G., Costa, M., and Eskay, R.L., Substance P-like immunoreactivity in nerves associated with the vascular system of guinea pigs. Neuroscience *7* (1982) 447–459.
29 Ghatei, M.A., Gu, J., Mulderry, P.K., Blank, M.A., Allen, J.M., Morrison, J.F.B., Polak, J.M., and Bloom, S.R., Calcitonin gene-related peptide (CGRP) in the female rat urogenital tract. Peptides *6* (1985) 809–815.
30 Gibbins, I.L., Furness, J.B., Costa, M., MacIntyre, I., Hillyard, C., and Girgis, S. A., Coexistence of calcitonin gene-related peptide, dynorphin and cholecystokinin in substance P-containing dorsal root ganglion neurons of the guinea pig. Neurosci. Lett. Suppl. 19–20 (1985) S.65.
31 Gibbins, I.L., Furness, J.B., Costa, M., MacIntyre, I., Hillyard, C.J., and Girgis, S., Co-localization of calcitonin gene-related peptide-like immunoreactivity with substance P in cutaneous, vascular and visceral sensory neurons of guinea pigs. Neurosci. Lett. *57* (1985) 125–130.
32 Gibson, S.J., Polak, J.M., Bloom, S.R., and Wall, P.D., The distribution of nine peptides in rat spinal cord with special emphasis on the substantia gelatinosa and area around the central (lamina X). J. comp. Neurol. *201* (1981) 65–79.
33 Gibson, S.J., Polak, J.M., Bloom, S.R., Sabate, I.M., Mulderry, P.K., Ghatei, M.A., McGregor, G.P., Morrison, J.F.B., Kelly, J.S., and Rosenfeld, M.G., Calcitonin gene-related peptide (CGRP) immunoreactivity in the spinal cord of man and eight other species. J. Neurosci. *4* (1984) 3101–3111.
34 Gibson, S.J., Polak, J.M., Anand, P., Blank, M.A., Morrison, J.F.B., Kelly, J.S., and Bloom, S.R., The distribution and origin of VIP in the spinal cord of six mammalian species. Peptides *5* (1984) 201–207.
35 Gibson, S.J., Polak, J.M., Anand, P., Blank, M.A., Yiangou, Y., Su, H.C., Terenghi, G., Katagiri, T., Morrison, J.F.B., Lumb, B.M., Inyama, C., and Bloom, S.R., A VIP/PHI-containing pathway links urinary bladder and sacral spinal cord. Peptides *7* (1986) 205–219.
36 Gu, J., Polak, M., Probert, L., Islam, K.N., Marangos, P.J., Mina, S., Adrian, T.E., McGregor, G.P., O'Shaughnessy, D.J., and Bloom, S.R., Peptidergic innervation of the human male genital tract. J. Urol. *130* (1983) 386–391.
37 Gu, J., Blank, M.A., Huang, W.H., Islam, K.N., McGregor, G.P., Christofides, N., Allen, J.M., Bloom, S.R., and Polak, J.M., Peptide-containing nerves in human urinary bladder. Urology *25* (1984) 353–357.
38 Gu, J., Polak, J.M., Blank, M.A., Terenghi, G., Morrison, J.F.B., and Bloom, S.R., The origin of VIP-containing nerves in the urinary bladder of rat. Peptides *5* (1984) 219–223.
39 Hökfelt, T., Schultzberg, M., Elde, R., Nilsson, G., Terenius, L., Said, S., and Goldstein, M., Peptide neurons in peripheral tissues including the urinary tract: immunohistochemical studies. Acta pharmac. toxic. *43* Suppl. II (1978) 79–89.
40 Hökfelt, T., Johansson, O., Ljungdahl, A., Lundberg, J.M., and Schultzberg, M., Peptidergic neurons. Nature *284* (1980) 515–521.
41 Holzer, P., Bucsics, A., and Lembeck, F., Distribution of capsaicin-sensitive nerve fibres containing immunoreactive substance P in cutaneous and visceral tissues of the rat. Neurosci. Lett. *31* (1982) 253–257.
42 Holzer-Petsche, U., and Lembeck, F., Systemic capsaicin treatment impairs the micturition reflex in the rat. Br. J. Pharmac. *83* (1984) 935–941.
43 Honda, C.N., Rethelyi, M., and Petrusz, P., Preferential immunohistochemical localization of vasoactive intestinal polypeptide (VIP) in the sacral spinal cord of the cat: Light and electron, microscopic observations. J. Neurosci. *5* (1983) 2183–2196.
44 Hoyes, A.D., and Barber, P., Degeneration of axons in the ureteric and duodenal nerve plexuses of the adult rat following in vivo treatment with capsaicin. Neurosci. Lett. *25* (1981) 19–24.
45 Hua, X.Y., Theodorsson-Norheim, E., Brodin, E., Lundberg, J.M., and Hökfelt, T., Multiple tachykinins (neurokinin A, neuropeptide K and substance P) in capsaicin-sensitive sensory neurons in the guinea pig. Reg. Pept. *13* (1985) 1–19.
46 Hua, X.Y., Saria, A., Gamse, R., Theodorsson-Norheim, E., Brodin, E., and Lundberg, J.M., Capsaicin induced release of multiple tachykinins (substance P, neurokinin A and eledoisin-like material) from guinea pig spinal cord and ureter. Neuroscience *19* (1986) 313–319.
47 Hua, X.Y., and Lundberg, J.M., Dual capsaicin effects on ureteric motility: low dose inhibition mediated by calcitonin gene-related peptide and high dose stimulation by tachykinins? Acta physiol. scand. *128* (1986) 453–465.
48 Inyama, C.O., Wharton, J., Su, H.C., and Polak, J.M., CGRP-immunoreactive nerves in the genitalia of the female rat originate from dorsal root ganglia T_{11}-L_3 and L_6-S_1: A combined immunocytochemical and retrograde tracing study. Neurosci. Lett. *69* (1986) 13–18.
49 Itoga, E., Kito, S., Kishida, T., Yanaihara, N., Ogawa, N., and Wakabayashi, I., Ultrastructural localization of substance P-, somatostatin-, Met-enkephalin- and beta-endorphin-like immunoreactivities in rat sensory ganglia and in cultured dorsal root ganglion cells. Acta histochem. cytochem. *12* (1979) 607.
50 Itoga, E., Kito, S., Kishida, T., Yanaihara, N., Ogawa, N., and Wakabayashi, I., Ultrastructural localization of neuropeptides in the rat primary sensory neurones. Acta histochem. cytochem. *13* (1980) 407–420.
51 Jancso, G., Hökfelt, T., Lundberg, J.M., Kirally, E., Halasz, N., Nilsson, G., Terenius, L., Rehfeld, J., Steinbusch, H., Verhofstad, A., Elde, R., Said, S., and Brown, M.J., Immunohistochemical studies on the effect of capsaicin on spinal and medullary peptide and monoamine neurons using antisera to substance P, gastrin/CCK, somatostatin, VIP, enkephalin, neurotensin and 5-hydroxytryptamine. Neurocytology *10* (1981) 963–980.
52 Jänig, W., and Morrison, J.F.B., Functional properties of spinal visceral afferents supplying abdominal and pelvic organs, with special emphasis on visceral nociception. Prog. Brain Res. *67* (1986) 87–114.
53 Jeftinija, S., Murase, K., Nedeljkov, V., and Randic, M., Vasoactive intestinal polypeptide excites mammalian dorsal horn neurons both in vivo and in vitro. Brain Res. *243* (1982) 148–164.
54 Jessell, T.M., and Iversen, L.L., Opiate analgesics inhibit substance P release from rat trigeminal nucleus. Nature *268* (1977) 549–551.
55 Jessell, T.M., Neurotransmitters and CNS disease: Pain. Lancet *2* (1982) 1084–1085.
55a Ju, G., Hökfelt, T., Fischer, J.A., Frey, P., Rehfeld, J.F., and Dockray, G.J., Does cholecystokinin-like immunoreactivity in rat primary sensory neurons represent calcitonin gene-related peptide? Neurosci. Lett. *68* (1986) 305–310.
56 Kawatani, M., Lowe, I., Nadelhaft, I., Morgan, C., and de Groat, W.C., Vasoactive intestinal polypeptide in visceral afferent pathways to the sacral spinal cord of the cat. Neurosci. Lett. *42* (1983) 311–316.
57 Kawatani, M., Lowe, I., Moossy, J., Martinez, J., Nadelhaft, I., Eskay, R., and de Groat, W.C., Vasoactive intestinal polypeptide (VIP) is localized to the lumbosacral segments of the human spinal cord. Soc. Neurosci. Abstr. *9* (1983) 294.
58 Kawatani, M., Kuo, D., and de Groat, W.C., Identification of neuropeptides in visceral afferent neurons in the thoracolumbar dorsal ganglia of the cat. Vth International Washington Spring Symposium, May 29–31, Washington, DC, 1985, Abstr. 156.
59 Kawatani, M., Erdman, S., and de Groat, W.C., Vasoactive intestinal polypeptide and substance P in afferent pathways to the sacral spinal cord of the cat. J. comp. Neurol. *241* (1985) 327–347.
60 Kawatani, M., Houston, M.B., Rutigliano, M., Erdman, S.L., and de Groat, W.C., Co-localization of neuropeptides in afferent pathways to the urinary bladder and colon: Demonstration with double color immunohistochemistry in combination with axonal tracing techniques. Soc. Neurosci. Abstr. *11* (1985) 145.
61 Kawatani, M., Rutigliano, M., and de Groat, W.C., Selective facilitatory effect of vasoactive intestinal polypeptide (VIP) on muscarinic firing in vesical ganglia of the cat. Brain Res. *336* (1985) 223–234.
62 Kawatani, M., Rutigliano, M., and de Groat, W.C, Vasoactive intestinal polypeptide produces ganglionic depolarization and facilitates muscarinic excitatory mechanisms in a sympathetic ganglion. Science *229* (1985) 879–881.
63 Kawatani, M., Nagel, J., and de Groat, W.C., Identification of neuropeptides in pelvic and pudendal nerve afferent pathways to the sacral spinal cord of the cat. J. comp. Neurol. *249* (1986) 117–132.
64 Keast, J.R., Furness, J.B., and Costa, M., Origins of peptide and norepinephrine nerves in the mucosa of the guinea pig small intestine. Gastroenterology *86* (1984) 637–644.
65 Keast, J.R., Furness, J.B., and Costa, M., Distribution of certain peptide-containing nerve fibres and endocrine cells in the gastrointestinal mucosa in five mammalian species. J. comp. Neurol. *236* (1985) 403–422.
66 Kim, J.H.K., Kim, S.U., and Kito, S., Immunocytochemical

demonstration of B-endorphin and B-lipotropin in cultured human spinal ganglion neurons. Brain Res. *304* (1984) 192–196.
67 Konishi, S., and Otsuka, M., Blockade of slow excitatory postsynaptic potential by substance P antagonists in guinea-pig sympathetic ganglia. J. Physiol., Lond. *361* (1985) 115–130.
68 Kumazawa, T., Sensory innervation of reproductive organs. Prog. Brain Res. *67* (1986) 115–131.
69 Kummer W., and Heym, C., Correlation of neuronal size and peptide immunoreactivity in the guinea-pig trigeminal ganglion. Cell Tissue Res. *245* (1986) 657–665.
70 Kuo, D., Nadelhaft, I., Hisamitsu, T., and de Groat, W.C., Segmental distribution and central projection of renal afferent fibers in the cat studied by transganglionic transport of horseradish peroxidase. J. comp. Neurol. *216* (1983) 162–174.
71 Kuo, D., Oravitz, J.J., Eskay, R., and de Groat, W.C., Substance P in renal afferent perikarya identified by retrograde transport of fluorescent dye. Brain Res. *323* (1984) 168–171.
72 La Motte, C., Pert, C.B., and Snyder, S.H., Opiate receptor binding in primate spinal cord, Distribution and changes after dorsal root section. Brain Res. *112* (1976) 407–412.
73 Larsen, J.J., Ottesen, B., Fahrenkrug, J., and Fahrenkrug L., Vasoactive intestinal polypeptide (VIP) in the male genitourinary tract: Concentration and motor effect. Inv. Urol. *19* (1981) 211–213.
74 Larsson, L.I., Fahrenkrug, J., and Schaffalitzky de Muckadell, O.B., Vasoactive intestinal polypeptide occurs in nerves of the female genitourinary tract. Science *197* (1977) 1374–1375.
75 Leah, J.D., Cameron, A.A., Kelly, W.L., and Snow, P.J., Coexistence of peptide immunoreactivity in sensory neurons of the cat. Neuroscience *16* (1985) 683–690.
76 Lee, Y., Takami, K., Kawai, Y., Girgis, S., Hillyard, C.J., MacIntyre, I., Emson, P.C., and Tohyama, M., Distribution of calcitonin gene-related peptide in the rat peripheral nervous system with reference to its coexistence with substance P. Neuroscience *15* (1985) 1227–1237.
77 Lundberg, J.M., Hökfelt, T., Anggard, A., Uvnas-Wallensten, K., Brimijoin, S., Brodin, E., and Fahrenkrug, J., Peripheral peptide neurons: Distribution, axonal transport, and some aspects on possible function, in: Neural Peptides and Neuronal Communication, pp. 25–36. Eds E. Costa and M. Trabucchi. Raven Press, New York 1980.
78 Lundberg, J.M., Saria, A., Theodorsson-Norheim, E., Brodin, E., Hua, X., Martling, C., Gamse, R., and Hökfelt, T., Multiple tachykinins in capsaicin-sensitive afferents: Occurrence, release and biological effects with special reference to irritation of the airways, in: Tachykinin Antagonists, pp. 159–169. Eds R. Hakanson and F. Sundler. Elsevier, Amsterdam 1985.
79 Maggi, C.A., and Meli, A., The role of neuropeptides in the regulation of the micturition reflex. J. auton. Pharmac. *6* (1986) 133–162.
80 Maggi, C.A., Santicioli, P., and Meli, A., The effects of topical capsaicin on rat urinary bladder motility in vivo. Eur. J. Pharmac. *103* (1984) 41–50.
81 Maggi, C.A., Santicioli, P., and Meli, A., Evidence for the involvement of endogenous substance P in the motor effects of capsaicin on the rat urinary bladder. J. Pharm. Pharmac. *37* (1985) 203–204.
82 Maggi, C.A., Santicioli, P., Giuliani, S., Abelli, L., and Meli, A., The motor effect of the capsaicin-sensitive inhibitory innervation of the rat ureter. Eur. J. Pharmac. *126* (1986) 333–336.
83 Maggi, C.A., Santicioli, P., Borsini, F., Giuliani, S., and Meli, A., The role of the capsaicin-sensivite innervation of the rat urinary bladder in the activation of micturition reflex. Arch. Pharmac. *332* (1986) 276–283.
84 Maggi, C.A., Santicioli, P., Giuliani, S., Furio, M., and Meli, A., The capsaicin-sensitive innervation of the rat urinary bladder: further studies on mechanisms regulating micturition threshold. J. Urol. *136* (1986) 696–700.
85 Maggi, C.A., Santicioli, P., Giuliani, S., Regoli, D., and Meli, A., Activation of micturition reflex by substance P and substance K: Indirect evidence for the existence of multiple tachykinin receptors in the rat urinary bladder. J. Pharmac. exp. Ther. *238* (1986) 259–266.
86 Matthews, M.R., and Cuello, A.C., Substance P-immunoreactive peripheral branches of sensory neurons innervate guinea pig sympathetic neurons. Proc. natn. Acad. Sci. USA *79* (1982) 1668–1672.
87 Mattiasson, A., Ekblad, E., Sundler, F., and Uvelius, B., Origin and distribution of neuropeptide Y-, vasoactive intestinal polypeptide- and substance P-containing nerve fibers in the urinary bladder of the rat. Cell Tissue Res. *239* (1985) 141–146.
88 Mo, N., and Dun, N.J., Vasoactive intestinal polypeptide facilitiates muscarinic transmission in mammalian sympathetic ganglia, Neurosci. Lett. *52* (1984) 19–23.
89 Mo, N., and Dun, N.J., Cholecystokinin octapeptide depolarizes guinea pig inferior mesenteric ganglion cells and facilitates nicotinic transmission. Neurosci. Lett. *64* (1986) 263–268.
90 Morgan, C., Nadelhaft, I., and de Groat, W.C., The distribution of visceral primary afferents from the pelvic nerve within Lissauer's tract and the spinal gray matter and its relationship to the sacral parasympathetic nucleus. J. comp. Neurol. *201* (1981) 415–440.
91 Morgan, C., and O'Hara, P., Electron microscopic identification of vasoactive intestinal polypeptide (VIP) in visceral primary afferent axons in the sacral spinal cord of the cat. Anat. Rec. *208* (1984) 121.
92 Morgan, C., de Groat, W.C., and Nadelhaft, I., The spinal distribution of sympathetic preganglionic and visceral primary afferent neurons which send axons into the hypogastric nerves of the cat. J. comp. Neurol. *243* (1986) 23–40.
93 Morgan, C., Nadelhaft, I., and de Groat, W.C., The spinal distribution of visceral primary afferent neurons which send axons into the lumbar colonic nerves of the cat. Brain Res. *398* (1986) 11–17.
94 Nadelhaft, I., and Booth, A.M., The location and morphology of preganglionic neurons and the distribution of visceral afferents from the rat pelvic nerve: A horseradish perioxidase study. J. comp. Neurol. *226* (1984) 238–245.
95 Nadelhaft, I., Morgan, C.W., and de Groat, W.C., Localization of the sacral autonomic nucleus in the spinal cord of the cat by the horseradish peroxidase technique. J. comp. Neurol. *193* (1980) 265–281.
96 Nadelhaft, I., Roppolo, J., Morgan, C., and de Groat, W.C., Parasympathetic preganglionic neurons and visceral primary afferents in monkey sacral spinal cord revealed following the application of horseradish peroxidase to pelvic nerve. J. comp. Neurol. *216* (1983) 36–52.
97 Neale, J.H., Barker, J.L., Uhl, G.R., and Snyder, S.H., Enkephalin-containing neurons visualized in spinal cord cell cultures. Science *201* (1978) 467–469.
98 Nohmi, M., Shinnick-Gallagher, P., Gean, P.W., Gallagher, J.P., and Cooper, C.W., Calcitonin and calcitonin gene-related peptide enhance calcium-dependent potentials. Brain Res. *367* (1986) 346–350.
99 Ottesen, B., Larsen, J.J., Fahrenkrug, J., Stjernquist, M., and Sundler, F., Distribution and motor effect of VIP in female genital tract. Am. J. Physiol. *240* (1982) E32–E36.
100 Polak, J.M., and Bloom, S.R., Localisation and measurement of VIP in the genitourinary system of man and animals. Peptides *5* (1984) 225–230.
101 Randic, M., and Miletic, V., Effect of substance P in cat dorsal horn neurons activated by noxious stimuli. Brain Res. *128* (1977) 164–169.
102 Roppolo, J.R., Nadelhaft, I., and de Groat, W.C., The organization of pudendal motoneurons and primary afferent projections in the spinal cord of the rhesus monkey revealed by horseradish peroxidase. J. comp. Neurol. *234* (1985) 475–488.
103 Rosenfeld, M.G., Mermod, J.J., Amara, S.G., Swanson, L.W., Sawchenko, P.E., Rivier, J., Vale, W.W., and Evans, R.M., Production of a novel neuropeptide encoded by the calcitonin gene via tissue-specific RNA processing. Nature *304* (1983) 129–135.
104 Salt, T.E., and Hill, R.G., Neurotransmitter candidates of somatosensory primary afferent fibers. Neuroscience *10* (1983) 1083–1103.
105 Santicioli, P., Maggi, C.A., and Meli, A., Functional evidence for the existence of a capsaicin-sensitive innervation in the rat urinary bladder. J. Pharm. Pharmac. *38* (1986) 446–451.
106 Santicioli, P., Maggi, C.A., and Meli, A., The effect of capsaicin pretreatment on the cystometrograms of urethane anesthetized rats. J. Urol. *133* (1985) 700–703.
107 Saria, A., Lundberg, J.M., Hua, X., and Lembeck, F., Capsaicin-induced substance P release and sensory control of vascular permeability in the guinea-pig ureter. Neurosci. Lett. *41* (1983) 167–172.
108 Saria, A., Ma, R.C., and Dun, N.J., Neurokinin A depolarizes neurons of the guinea pig inferior mesenteric ganglia. Neurosci. Lett. *60* (1985) 145–150.
109 Sasek, C.A., Seybold, V.S., and Elde, R.P., The immunohistochemical localization of nine peptides in the sacral parasympathetic nucleus and the dorsal gray commissure in rat spinal cord. Neuroscience *12* (1984) 855–873.
110 Schroder, H.D., Localization of CCK-like immunoreactivity in the rat spinal cord with particular reference to the autonomic innervation of the pelvic organs. J. comp. Neurol. *217* (1983) 176–186.
111 Schultzberg, M., Hökfelt, T., Lundberg, J.M., Fuxe, K., Mutt, V., and Said, S., Distribution of VIP neurons in the peripheral and central nervous system. Endocr. japon. *1* (1980) 23–30.
112 Senba, E., Shiosaka, S., Hara, Y., Inagaki, S., Sakanaka, M., Takatsuki, K., Kaway, Y., and Tohyama, M., Ontogeny of the peptidergic

system in the rat spinal cord: immunohistochemical analysis. J. comp. Neurol. *208* (1982) 54–66.
113 Sharkey, K.A., Williams, R.G., Schultzberg, M., and Dockray, G.J., Sensory substance P-innervation of the urinary bladder: Possible site of action of capsaicin in causing urine retention in rats. Neuroscience *10* (1983) 861–868.
114 Su, H.C., Wharton, J., Polak, J.M., Mulderry, P.K., Ghatei, M.A., Gibson, S.J., Terenghi, G., Morrison, J.F.B., Ballesta, J., and Bloom, S.R., Calcitonin gene-related peptide immunoreactivity in afferent neurons supplying the urinary tract: combined retrograde tracing and immunocytochemistry. Neuroscience *18* (1986) 727–747.
115 Sweetnam, P.M., Neale, J.H., Baker, J.L., and Goldstein, A., Localization of immunoreactive dynorphin in neurons cultured from spinal and dorsal root ganglia. Proc. natn. Acad. Sci. USA *79* (1982) 6742–6746.
116 Theodorsson-Norheim, E., Hua, X., Brodin, E., and Lundberg, J.M., Capsaicin treatment decreases tissue levels of neurokinin A-like immunoreactivity in the guinea pig. Acta physiol. scand. *124* (1985) 129–131.
117 Thor, K., Kawatani, M., and de Groat, W.C., Plasticity in the reflex pathways to the lower urinary tract of the cat during postnatal development and following spinal cord injury, in: Development and Plasticity of the Mammalian Spinal Cord, Fidia Research Series, vol. III, pp. 65–81. Eds M. Goldberger, A. Gorio and M. Murray. Liviana Press, Padova, Italy 1986.
118 Traurig, H., Saria, A., and Lembeck, F., Substance P in primary afferent neurons of the female rat reproductive system. Naunyn Schmiedeberg's Arch. Pharmac. *326* (1984) 343–346.
119 Traurig, H., Papka, R., and Urban, L., Origin of peptide containing nerves in the female rat reproductive system. Anat. Rec. *211* (1985) 199A–200A.
120 Tsunoo, A., Konishi, S., and Otsuka, M., Substance P as an excitatory transmitter of primary afferent neurons in guinea-pig sympathetic ganglia. Neuroscience 7 (1982) 2025–2037.
121 Tuchscherer, M.M., and Seybold, V.S., Immunohistochemical studies of substance P, cholecystokinin-octapeptide and somatostatin in dorsal root ganglia of the rat. Neuroscience *14* (1985) 593–605.
122 Ueyama, T., Mizuno, N., Nomura, S., Konishi, A., Itoh, K., and Arakawa, H., Central distribution of afferent and efferent components of the pudendal nerve in cat. J. comp. Neurol. *222* (1984) 38–46.
123 Weihe, E., Hartschuh, W., and Weber, E., Prodynorphin opioid peptides in small somatosensory primary afferents of guinea pig. Neurosci. Lett. *58* (1985) 347–352.
124 Wiesenfeld-Hallin, Z., Hökfelt, T., Lundberg, J.M., Forssmann, W.G., Reinecke, M., Tschopp, F.A., and Fischer, J.A., Immunoreactive calcitonin gene-related peptide and substance P coexist in sensory neurons to the spinal cord and interact in spinal behavioral responses of the rat. Neurosci. Lett. *52* (1984) 199–204.
125 Yaksh, T.L., Jessell, T.M., Gamse, R., Mudge, A.W., and Leeman, S.E., Intrathecal morphine inhibits substance P release from mammalian spinal cord in vivo. Nature *286* (1980) 155–157.
126 Yaksh, T.L., Abay, E.O., and Go, V.L.W., Studies on the location and release of cholecystokinin and vasoactive intestinal polypeptide in rat and cat spinal cord. Brain Res. *242* (1982) 279–290.
127 Yokokawa, K., Sakanaka, M., Shiosaka, S., Tohyama, M., Shiotani, Y., and Sonoda, T., Three-dimensional distribution of substance P-like immunoreactivity in the urinary bladder of the rat. J. neural. Transm. *63* (1985) 209–222.

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Neuropeptides and the microcircuitry of the enteric nervous system

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Summary. The discovery of neuropeptides in enteric neurons has revolutionized the study of the microcircuitry of the enteric nervous system. From immunohistochemistry, it is now clear that some individual enteric neurons contain several different neuropeptides with or without other transmitter-specific markers and that these markers occur in various combinations. There is evidence from experiments in which nerve pathways are interrupted that populations of enteric neurons with different combinations of markers have different projection patterns, sending their processes to distinct targets using different routes. Correlations between the neurochemistry of enteric neurons and the types of synaptic inputs they receive are also beginning to emerge from electrophysiological studies. These findings imply that enteric neurons are chemically coded by the combinations of peptides and other transmitter-related substances they contain and that the coding of each population correlates with its role in the neuronal pathways that control gastrointestinal function.
Key words. Neuropeptides; enteric nervous system; intestine.

The mammalian gastrointestinal tract contains about as many neurons as the spinal cord[28]. These intrinsic (enteric) neurons, along with the processes of sympathetic, parasympathetic and sensory neurons supplying the gut, and enteric glial cells, make up the enteric nervous system (ENS), which is now generally classified as a third division of the autonomic nervous system. The cell bodies of the enteric neurons are grouped into ganglia in two main plexuses: the submucous plexus, discovered by Meissner in 1857[52], in the loose connective tissue of the submucosa, and the myenteric plexus, described by Auerbach in 1864[1], which lies between the longitudinal and circular layers of the mucularis externa. Non-ganglionated plexuses, which are continuous with the main ganglionated plexuses, supply the muscle layers, the mucosa and blood vessels.

The ENS influences or controls a variety of functions, including movement of digesta along the gastrointestinal tract, gastric acid secretion, transport of water and electrolytes, release of gastrointestinal hormones, and blood flow[29, 30]. Enteric reflexes persist even when the gut is disconnected from the central nervous system (e.g., Bayliss and Starling[2, 3]). Hence, the ENS must contain several different functional types of neurons, namely motor neurons to the muscle, secretomotor and vasomotor neurons, interneurons and sensory neurons. Futhermore, these neurons must be arranged in an orderly fashion to form circuits that govern the different enteric reflexes. The need to unravel the internal circuitry of the ENS was recognized early, but this analysis was frustrated until the past decade by the inadequacy of neurohistological and pathway tracing techniques. Since 1975, however, our knowledge of neuronal pathways in the ENS has increased significantly because of the discovery of neuropeptides in enteric neurons and because of technical advances, including light and electron microscopical methods for immunohistochemistry on whole mount preparations of separated gut layers, techniques for lesioning enteric nerve pathways, and correlated physiological, pharmacological and electrophysiological studies. The ways in which these devel-