Experientia 40 (1984), Birkhäuser Verlag, CH-4010 Basel/Switzerland

Comparative distribution of vasoactive intestinal polypeptide (VIP), substance P and PHI in the enteric sphincters of the cat

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Summary. In the feline gastrointestinal tract, the neuropeptides, substance P, VIP and PHI were investigated by specific radioimmunoassays and immunocytochemistry. The concentrations of all 3 peptides and the level of peptidergic innervation were significantly less in the anal sphincter than elsewhere, whereas no significant differences were seen between other sphincter and non-sphincter regions.

No major anatomical features appear to distinguish 'non-sphincter' from 'sphincter' smooth muscle in the GI tract nor to explain the different physiological properties of these 2 tissue types. However, in vitro, pharmacological studies have revealed on human and other mammalian tissue clear differences in the nature of the responses of the smooth muscle of the sphincters as compared to the rest of the gut[1,2]. These investigations have demonstrated, for example, that gut sphincters respond differently to adrenergic and cholinergic stimuli. One theory is that the many non-adrenergic, non-cholinergic neurones present in the intrinsic plexus of the gut wall[3], may specifically alter the effect on gut smooth muscles of these 2 neurotransmitters.

Substance, P, vasoactive intestinal polypeptide (VIP) and PHI (a peptide with histidine and isoleucine at its amino- and carboxy-terminals respectively) are major representatives of the many peptides which have been identified in non-cholinergic, non-adrenergic gut neurones[4-6]. They are thought to be neurotransmitters or neuromodulators and have specific biological actions including effects on gut motility[7]. VIP relaxes isolated gut smooth muscle[8,9] and has been considered the inhibitory mediator in the neural control of gut motility[10]. Substance P potently stimulates it[11,12]. This may be a direct action on muscle cells as well as a local modulation of cholinergic and adrenergic transmission[13]. PHI is a recently discovered neuropeptide[14] which has both structural homology with, and a similar distribution to, VIP. The peptidergic innervation of the enteric sphincters has not been extensively studied and, if different from that seen throughout the rest of the gut might underlie the characteristic functional differences seen in the sphincteric smooth muscle.

The distributions of VIP, substance P, and PHI, were investigated along the feline gastrointestinal tract, both by specific and sensitive radioimmunoassays, and by immunocytochemistry.

Segments of whole gut wall thickness, were carefully dissected out in the region of the cardiac and pyloric sphincters, the ileal-caecal valve and the anal sphincter. For each, serial 1-cm segments were taken immediately proximal to the sphincter, from the sphincter itself and, immediately distal to it. Representative segments were also taken from areas more remote from the sphincters; oesophagus, fundus, antrum, duodenum, ileum, colon and rectum. Each sample was divided and half extracted for radioimmunoassay of peptide content and half processed for immunocytochemistry.

For radioimmunoassay each piece of tissue was extracted in 0.5 M acetic acid. The tissue was first chopped into pieces of approximately 100 mg and then added to the boiling acid in polypropylene tubes which were held in a boiling water bath for 10 min. The extracts were then stored at −20 °C until assay. Aliquots of each extract (10 µl and 1 µl) were assayed in duplicate for each of the 3 peptides. Each assay has been recently described[6,15] and the table summarizes their details.

Pieces of whole gut wall, measuring no more than 1 × 1 cm, were fixed by immersion in 0.4% p-benzoquinone in phosphate buffered saline (PBS) (0.01 M, pH 7.1–7.4) for 2 h at 4 °C[16]. Fixed tissue was thoroughly rinsed in 7% sucrose in PBS and 10-µm sections were cut in a cryostat at −20 °C. The sections were immunostained by indirect immunofluorescence[17] using antibodies to VIP, substance P or PHI. These were applied, at dilutions of 1:2000, 1:1000 and 1:400 respectively, and left overnight at 4 °C. The 2nd layer of fluorescein conjugated goat anti-rabbit gamma globulin was applied at 1:100 for 1 h. Sections were mounted in PBS: glycerine (1:9) and examined under a Leitz fluorescence microscope.

The figure 1 shows the levels (pmol/g) of each peptide along the feline alimentary tract. They were all present in substantial quantities throughout the gut including the sphincter regions with particularly high levels of VIP and PHI in the more distal bowel.

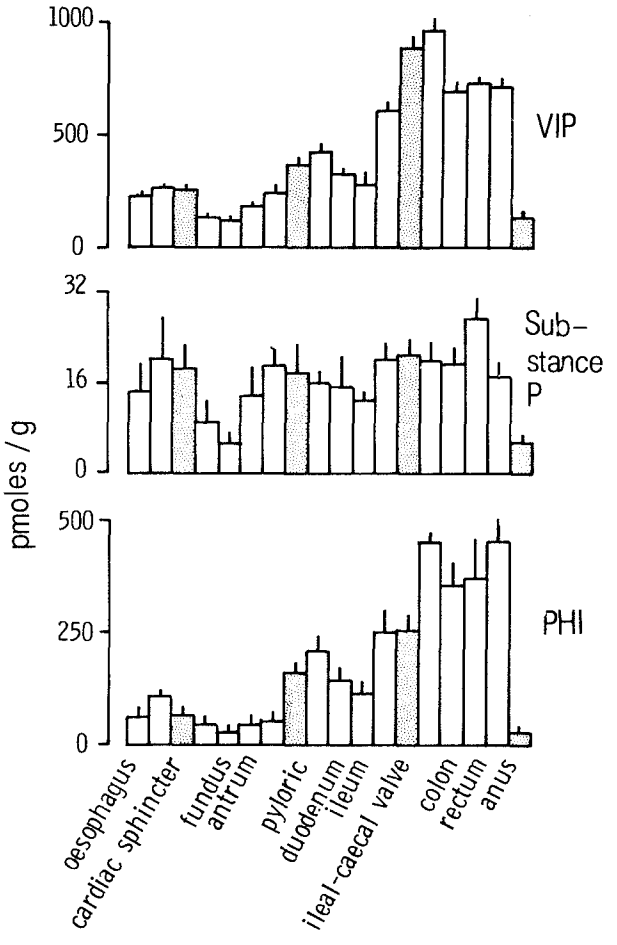

Figure 1. The mean concentration (± SEM) of VIP, substance P and PHI in the feline gut in pmol/g. The values for the cardiac and pyloric sphincters, ileal-caecal valve and the anal sphincter are shown (shaded). The values for the segments 1 cm proximal and 1 cm distal to the sphincter region are represented by the columns to the right and left respectively of the shaded columns. The other columns indicate the mean values for regions (as labeled) more remote from the sphincter areas.

In agreement with these results, immunocytochemistry localized the 3 neuropeptides to nerve fibers in all areas examined. Fibers containing VIP were the most abundant and those containing substance P the least frequently observed. All 3 types of peptide containing nerves were present in each layer of the gut wall. In the longitudinal and circular muscle coats and in the muscularis mucosa the peptide containing nerves ran parallel to the muscle fibers and were closely entwined with them. In the mucosa all types of nerves ran both along the base of the mucosa and projected up to the lamina propria. Those containing PHI and VIP showed frequent close association with the mucosal epithelium. Blood vessels were found in all areas surrounded by the peptide-containing fibers, particularly those with PHI and VIP. A significant proportion of the submucosal nerve cells contained VIP. PHI-immunoreactive cells were also found in lesser numbers, but no cell bodies showed substance P-immunoreactivity. No obvious increase in the number of fibers could be seen in the different levels of gut, except in the pyloric sphincter. In this region, VIP nerves were relatively more numerous along the inner edge of the circular muscle coat, a region previously termed the deep muscular plexus (fig. 2A).

The most striking feature of these results was the comparatively very low levels of all three peptides in the anal sphincter. For each peptide the concentration in the anal spincter was very significantly less ($p < 0.001$) than that in the 1-cm segment of bowel taken immediately proximal; VIP being 82% less in the spincter while the corresponding figures for substances P and PHI were 77 and 94% respectively. These differences cannot be fully accounted for by the inclusion, in the extracts, of skeletal muscle from the external sphincter where only a few peptide nerves were seen, in association with blood vessels. In the internal anal sphincter, not only were fewer fibers found but also the frequency of neuropeptide containing ganglion cells was reduced. This pattern was not seen in the other sphincter regions where, for all 3 peptides, there was no significant difference in the concentrations at the sphincter itself as compared to the adjacent proximal and distal non-sphincter tissue. Across the cardiac and pyloric sphincters and the ileal-caecal valve there is a gradient of both VIP and PHI concentration but the differences were not statistically significant. They were similar to those reported previously for VIP alone[18].

An absence of enteric ganglia from the internal anal sphincter has been reported[19] in many mammalian species, including the cat. There also appears to be a virtual absence of processes

Details of radioimmunoassays

	Substance P	VIP	PHI
Immunogen			
Hapten	Substance P	Natural porcine	Natural porcine
Carrier	BSA	BSA	BSA
Coupling reagent[15]	Glutaraldehyde	CDI	Glutaraldehyde
Antibody			
Specificity	C-terminal	Mid to C-terminal	Mid to C-terminal
Final dilution	1 in 8,000	1 in 320,000	1 in 10,000
Reaction other mammalian peptides	None	None (< 0.1% PHI)	None (< 0.1% VIP)
Radio-iodination			
Method	Chloramine-T oxidation	Chloramine-T oxidation	Iodogen
^{125}I-tracer	tyr^{8}-substance P	Natural porcine VIP	Natural porcine PHI
Assay standard	Substance P	Natural porcine VIP	Natural porcine PHI
Assay sensitivity (fmoles/assay tube − 95% confidence)	0.3	0.4	2.0

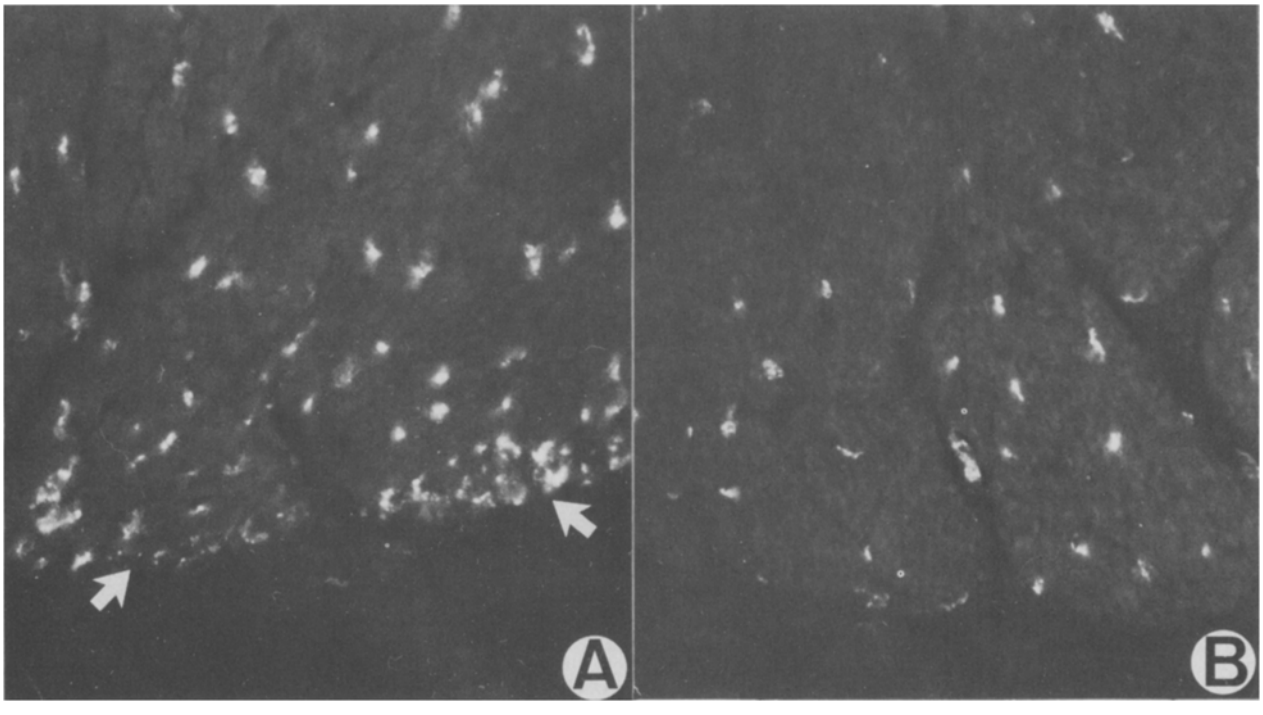

Figure 2. *A* VIP immunoreactive nerve fibers in a cross-section of the circular muscle coat of the pyloric sphincter. Note the slight increase in the density of innervation along the inner edge of the muscle (arrow). *B* The circular muscle coat in the region of the internal anal sphincter (cross-section), containing fewer VIP-immunoreactive fibers than in A. × 265.

from neurones intrinsic to the bowel, which is associated with an increased extrinsic, mainly adrenergic innervation.

VIP, substance P and PHI containing nerves[4,5,16] are major elements of the enteric nervous system and these results from the cat suggest that, in the anal sphincter, there is a reduced peptidergic innervation which is not seen in the other gut sphincters. The reduced level of intrinsic, including peptide-containing (fig. 2B), innervation in the internal anal sphincter provides a region of tonic contraction for retention of feces. This contraction is, presumably, overridden by nerves mediating the central nervous input and local autonomic reflexes to enable defecation.

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0014-4754/84/050469-03$1.50 + 0.20/0

Aspects of interastrocytic gap junctions in blood-brain barrier in the experimental penumbra area, revealed by transmission electron microscopy and freeze-fracture

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Summary. Interastrocytic gap junctions in the blood-brain barrier of the experimental penumbra area were studied in the cat caudate nucleus 1 h after ischemia. Transmission electron microscopy and freeze-fracture studies revealed only slight changes in gap junctions between astrocytes, indicating that these junctions are very resistant to hypoxia.

The penumbra area[2] is a peri-infarct zone where the cerebral tissue can survive structurally but does not function electrophysiologically. These dormant neurons would benefit most from management strategies that increase blood flow in the area surrounding the infarct. In an experimental model of focal and selective cerebral ischemia[3], the penumbra area has been determined ultrastructurally after 1 h of ischemia (manuscript submitted for publication). Ultrastructural changes in the blood-brain barrier of the penumbra area, especially the endothelial cylinder, have been studied in different postischemic periods. Tissue functions depend on intact cellular membranes; an early response to an insult such as ischemia might well be reflected by molecular changes in membrane components[4]. Although many ultrastructural studies have described ischemic neurons there is no published information concerning molecular membrane structure in cerebral tissue after ischemia. The aim of the present study was to examine with transmission electron microscopy and freeze-fracture technique the gap junctions between astrocytic foot processes in the penumbra area after 1 h of circulatory arrest.

Material and methods. Experiments were performed in 5 cats weighing 2.5–3.5 kg, anesthetized with i.v. injection of pentobarbital (30 mg/kg) and 0.4 mg atropine. An endotracheal tube was installed, and a Harward respirator was used to maintain blood gases and pH within physiological limits. The anterolateral group of penetrating lenticulo-striated arteries arising from the M1 segment of middle cerebral artery and orbitofrontal artery origin were exposed through a transorbital approach and were occluded with bipolar coagulation using the lower current setting. The ischemic area produced was localized in the anterior part of the internal capsule and head of the caudate nucleus[3]. After 60 min of ischemia, the cats were fixed by retrograde perfusion through the abdominal aorta with a mixture of glutaraldehyde and paraformaldehyde[5]. After perfusion-fixation, the brain was left in situ for 1 h to eliminate dark cells, hydropic cells, and artifactitious perivascular spaces[6]. After dissection, samples of caudate nucleus were selected for transmission electron microscopy and the freeze-fracture technique. For electron microscopy, small tissue blocks of caudate nucleus were postfixed, after a buffer rinse, in 1% OsO_4 in 0.1 M sodium cacodylate buffer for 1 h. The tissues were then dehydrated in a graded series of ethanols, and embedded in Epon 812, with a transitional step in propylene oxide. 1-μm-thick toluidine blue stained sections from different caudate nucleus regions were selected for examination under the EM. Thin sections of silver-to-grey interference color were cut with a diamond knife mounted on a LKB ultratome. Sections were stained with uranyl acetate and lead ci-