

Changes in vasoconstrictor and vasodilator neurotransmitters in nerves supplying arterioles in developing colorectal polyps

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Abstract

Objective To examine the changes that occur in the immunohistochemistry of vasoconstrictor and vasodilator transmitters in nerves supplying early and advanced colorectal polyps.

Subjects and methods We studied the perivascular innervation of submucosal arterioles of colorectal polyps ($n = 18$) and the innervation of the epithelial layer of polyps compared to normal controls ($n = 8$), using immunohistochemical markers for the neurotransmitters; noradrenaline (NA) (marker used; tyrosine hydroxylase (TH)), neuropeptide Y (NPY), vasoactive intestinal polypeptide (VIP), substance P (SP), and calcitonin gene-related polypeptide (CGRP). (Advanced polyps; villous adenomas > 1.5 cm, polyps with severe dysplasia or partial carcinoma).

Results In submucosal arterioles there was a progressive decrease from controls through early polyps to advanced polyps in TH and NPY perivascular immunoreactivity

($P < 0.015$ for both). VIP and SP immunoreactivity was higher in early polyps compared to controls, but markedly reduced in advanced polyps ($P < 0.05$ for VIP). Sparse CGRP immunoreactivity was present in polyps only. Neural VIP and SP immunoreactivity in the lamina propria of polyp mucosa was more intense than in controls.

Conclusion There is a decrease in vasoconstrictor neurotransmitters NPY and NA (shown by TH) around submucosal arterioles of both early and advanced polyps, but an increase in the vasodilator neurotransmitters, particularly VIP, in early colorectal polyps. These results suggest a predominantly vasodilatory neural influence in early polyps, perhaps indicating a mechanism that maintains polyp growth.

Keywords Colorectal polyps, neuropeptide Y, tyrosine hydroxylase, vasoactive intestinal polypeptide, calcitonin gene-related polypeptide, substance P, innervation

Introduction

The growth of colorectal adenomatous polyps, the precursors of colorectal cancer, can be slowed down by modulating their blood supply. Inhibition of angiogenesis in adenomas by agents such as prostaglandin synthase inhibitors (aspirin, sulindac) are reported to be effective [1,2]. These drugs appear to cause regression not only in familial cases of polyposis, but also in larger polyps and sporadic polyps [2–4].

In this study, we considered the feeder vessels of colorectal polyps, as opposed to the new vessels. Both the normal colonic mucosa and polyps are supplied by

arterioles, which branch at the mucosa/submucosal border [5,6]. These feeder submucosal arterioles are the final resistance vessels in the enteric circulation and are endowed with the richest innervation of the microcirculation. Although within colorectal cancer perivascular nerves are mainly absent, the feeder vessels in the tissue adjacent to the tumour are innervated [7]. Using specific markers, we have previously shown that in submucosal tissue adjacent to Dukes' A, B and C colorectal cancers, there is a decrease in autonomic perivascular nerves and the innervation profile is different when compared to normal submucosa [8].

In this study, we examined the immunoreactivity to tyrosine hydroxylase (TH), neuropeptide Y (NPY) (sympathetic nerve markers), vasoactive intestinal peptide (VIP, enteric nerve marker), substance P (SP) and calcitonin gene related peptide (CGRP) (sensory-motor nerve markers) in the feeder submucosal vessels of

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colorectal polyps and the lamina propria of polyp mucosa. The study was designed to examine the differences between polyps and controls with a view to determining whether therapeutic manipulation of the blood supply of colorectal polyps might be a feasible approach to reducing tumour progression.

Patients and methods

Control and polyp tissue was taken from 16 patients undergoing colonoscopy or surgical resection of the large bowel (consent given). The mean age of the 16 patients was 63.0 years (range 45–78 years; 9 females, 7 males). Eighteen polyps (12 early, 6 advanced, i.e. those villous adenomas > 1.5 cm, polyps with associated malignancy or severe dysplasia) were harvested. Normal control samples of mucosa and submucosa were taken at least 5 cm away from the diseased areas. All operative specimens were taken in the longitudinal axis.

For light microscopy, the specimens were placed in 4% paraformaldehyde in 0.1 M phosphate buffered saline (PBS) and fixed overnight. The tissues were then placed overnight in 7% sucrose in PBS and frozen in liquid nitrogen (-196°C). Sections (10 µm) were cut and air-dried onto gelatin-coated slides for immunohistochemistry and adjacent sections were taken for haematoxylin and eosin staining. For immunohistochemistry, the slides were incubated overnight with polyclonal rabbit antibody to tyrosine hydroxylase (TH) (Affiniti, Exeter, UK), neuropeptide Y (NPY) (Biogenesis, Poole, UK), vasoactive intestinal polypeptide (VIP) (Incstar, Woking, UK), substance P (SP) (Genosys, Cambridge, UK), or calcitonin gene-related peptide (CGRP) (Affiniti) followed by incubation for one hour with donkey anti-rabbit biotinylated species-specific antibody (Amersham Life Science, Amersham, UK). Immunostaining was performed using streptavidin fluorescein (Amersham Life Science).

Positive controls were used from normal samples taken from the specimens at least 5 cm from diseased areas in order to ensure that changes observed were related to the pathology studied as opposed to factors affecting the colon in general. For negative controls the primary antibody was omitted.

The total number of submucosal arterioles supplying the mucosa or the polyps were counted as well as the number of submucosal arterioles showing perivascular innervation (3 fields per section, $\times 250$ magnification). Parallel assessment by an independent observer yielded 96% interobserver correlation. The results were expressed as percentages and means of percentages and subjected to analysis of variance followed by Tukey-Kramer multiple comparison test where appropriate.

Results

Submucosal arterioles

The mean total number of arterioles was 6.5 for the controls obtained from the tumour resection specimens and 6.1 for the polyps (3 fields, $\times 250$ magnification). Both perivascular and paravascular nerves were present around the vessels.

We analysed our data by dividing polyps into early ($n = 12$) and advanced ($n = 6$) groups, and comparing with controls. Advanced polyps were defined as villous adenomas over 1.5 cm, polyps with severe dysplasia or partially replaced by carcinoma [9,10].

Overall, there was a progressive decrease from controls through early polyps to advanced polyps in perivascular immunoreactivity to TH (controls 81.3%, early polyps 49%, advanced polyps 18.7%; $P < 0.01$), and NPY (97.1%, 85.6%, 61.3%; $P < 0.015$) (Table 1).

Immunoreactivity to VIP and SP was higher in early polyps compared to controls (83.9% vs 51.3%; $P < 0.05$ and 50.8% vs 44.3%, respectively) but markedly reduced in advanced polyps (14.2% ($P < 0.01$) and 12.4%, respectively). Perivascular CGRP was usually absent in normal tissue, with a few immunoreactive nerves found in all polyps (Table 1).

Specifically, looking at the polyp groups; advanced polyps ($n = 5$) in comparison with early polyps ($n = 12$) showed a marked decrease in arteriolar perivascular immunoreactivity to TH, NPY (Fig. 1), VIP and SP; CGRP immunoreactivity ($n = 6$) was similar in both groups of polyps (Table 1).

Mucosa

In the normal mucosal layer, nerve fibres were present in the lamina propria coursing towards the apex of the colonic glands, and were frequently found in close association with the cells of the glands. The intensity of these fibres immunoreactive to VIP, SP and NPY was high. In samples where the glands were cut in transverse section, fine SP fibres were visualized forming a hexagonal pattern around the colonic cells. Similarly NPY immunoreactive fibres were seen in close apposition to the cells. The cytoplasm of colonic cells throughout the glands was immunoreactive to SP, but not to the other neurotransmitters.

Examination of the lamina propria of polyps revealed increased neural immunoreactivity to VIP and SP compared with normal controls. The VIP immunoreactivity was high throughout the depth of the periglandular tissue and at the base near the muscularis propria (Fig. 2). Immunoreactivity was intense for SP within the lamina

	Colorectal polyps					
	Normal tissue		Early		Advanced†	
	%	n	%	n	%	n
TH	81.3 ± 10.8	(8)	49.0 ± 11.6	(12)	18.7 ± 8.3**	(5)
NPY	97.1 ± 2.9	(7)	85.6 ± 6.6	(12)	61.3 ± 16.8*	(5)
VIP	51.3 ± 8.7	(8)	83.9 ± 4.1*	(12)	14.2 ± 8.7***‡	(5)
SP	44.3 ± 16.6	(7)	50.8 ± 10.7	(12)	12.4 ± 8.1	(5)
CGRP	0.0	(7)	16.1 ± 9.2	(12)	12.0 ± 12.0	(6)

Values are shown as mean % ± SEM (no.). TH, tyrosine hydroxylase; NPY, neuropeptide Y; VIP, vasoactive intestinal polypeptide; SP, substance P; CGRP, calcitonin gene-related peptide. †Advanced polyps were defined as villous adenomas > 1.5 cm, or polyps with severe dysplasia or malignant involvement. *P < 0.05, **P < 0.01 vs normal tissue; ‡P < 0.05 vs early polyps.

Table I Number of arterioles with perivascular immunoreactivity to tyrosine hydroxylase, neuropeptide Y, vasoactive intestinal polypeptide, substance P and calcitonin gene-related peptide.

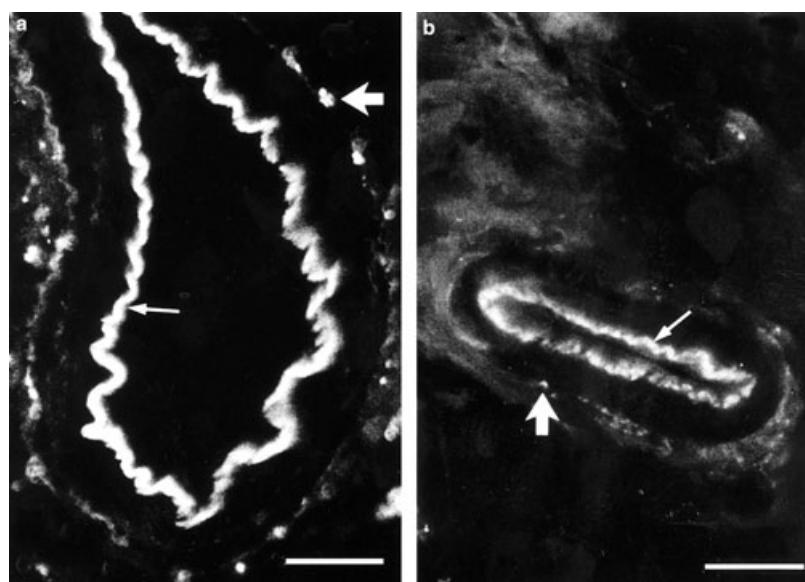


Figure 1 NPY-immunoreactivity in perivascular nerves of control tissue and benign polyps. Immunoreactivity to NPY (large arrows) is more intense in (a) controls relative to (b) benign polyps. Note small arrows indicating autofluorescence of the intima. Bar = 50 μm.

propria of many polyps with fibrillar appearance similar to normal controls but often more intense. In some samples, the muscularis mucosa immunoreacted well to CGRP. Immunoreactivity to TH was present in a few polyps within the muscularis mucosa but rarely was this evident elsewhere within the lamina propria. There did not appear to be any change from normal in NPY immunoreactivity. The colonic epithelial cells showed diffuse cytoplasmic immunoreactivity to SP in the majority of polyps.

Discussion and Conclusions

The normal colonic mucosa is supplied by a rich network of capillaries that form a regular pattern around the glandular elements [11]. These vessels issue from arterioles in the submucosa that branch at the submucosa/mu-

cosa border. The submucosal arterioles are the final vessels of resistance in the enteric circulation and are endowed with a rich supply of perivascular nerves. Studies of benign and malignant tumours both in the experimental situation in animal models, and in the human colon reveal that the tumour circulation in polyps is linked to the adjacent submucosa via these submucosal arterioles [6,12].

In this study, in the submucosa of the polyps, as opposed to the main polyp body itself, there was no increase in the number of arterioles compared to controls. This suggests that the arterioles supplying polyps are mainly pre-existing vessels. In a previous study [8], the mean number of submucosal arterioles in tissue adjacent to carcinomas was similar to that in normal control submucosa; it would seem therefore that the

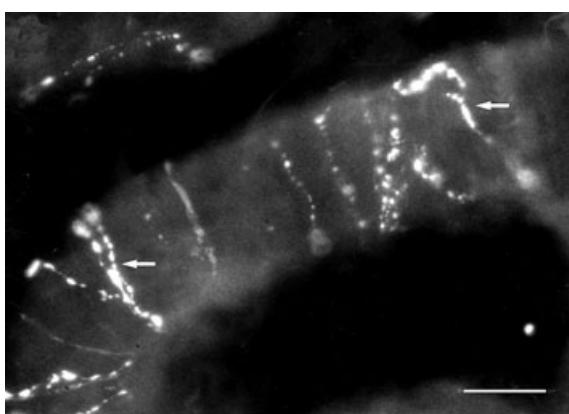


Figure 2 Neural immunoreactivity to VIP around submucosal vessels in an early polyp. Immunoreactivity is intense in perivascular nerves (arrow). Bar = 20 μ m.

density or count of arterioles in the normal tumour-associated tissue is not of prognostic significance. This differs from the situation in the polyp mass itself where the density of vessels appeared to be increased in polyps compared with normal mucosa [13].

Malignant tumour blood vessels are rarely innervated; usually, solid tumours such as colorectal carcinoma show no perivascular innervation within the tumour mass, whilst haemangiomas may be innervated [7,14]. In the tissue adjacent to colorectal cancer, as in normal colon, submucosal arterioles have the highest density of nerves compared with other microvessels [7]; these arterioles supply the tumour as it advances. In a previous work we found a decrease in the innervation of arterioles in the submucosa adjacent to Duke's A, B and C colorectal cancers [8].

The present study has shown that levels of perivascular immunoreactivity in arterioles supplying colorectal polyps differed from controls. Immunoreactivity to TH and NPY vasoconstrictor substances was decreased in arterioles supplying polyps. The difference between the percentage of arterioles with perivascular immunoreactivity observed between early polyps and advanced polyps is of interest, since it parallels the difference seen between normal submucosal arterioles and those supplying carcinomas in our previous study [8]. This led us to formulate the hypothesis that nerve loss in submucosal tissue adjacent to cancers was the result of tumour-released factors [8]. In this study, the changes in innervation of submucosa adjacent to advanced colorectal polyps, suggest that polyps acquire the ability to affect the sympathetic perivascular nerves in their progression towards becoming carcinomas.

VIP is a potent vasodilator in the gut [15]. The high levels of VIP immunoreactivity detected in submucosal

arterioles of early polyps suggests that there is a major vasodilatory influence early in the development of colorectal tumours. The vasodilatory influence is also maintained by an increase in SP and CGRP and a decrease in the vasoconstrictors TH and NPY around the arterioles of the submucosa of polyps. The immediate effect of arteriolar dilatation is an increase in local blood supply, as demonstrated in the bowel of human volunteers infused with SP [16]. The increase in blood supply would be to the advantage of the polyp, maintaining its growth. Recently, VIP was reported to stimulate the expression of vascular endothelial growth factor, which would further maintain the local blood vessel network [17].

VIP has also been implicated in the nerved-mediated atropine-resistant electrolyte and mucus secretion by the mucosa of the large bowel [18,19]. VIP immunoreactivity was higher in the lamina propria of polyps compared with normal mucosa; this suggests that VIP may lead to the increase in fluid and electrolyte secretion, and mucus secretion observed in these polyps, especially the villous types [20]. In these cases, the loss of fluids and electrolytes may be severe and patients may present with severe hypokalaemia [14,21]. Blockers of VIP-induced vasodilation and hypersecretion may therefore have a therapeutic role.

The results in this study suggest that the use of VIP antagonists at the early stage of polyp development may delay the transformation to malignancy.

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