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Relations between Leu^5-enkephalin- (LENK) and VIP-immunoreactive nerve fibres during human drug-resistant colitis. A case study

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The double immunofluorescence technique was used to examine the distribution and interrelationship between LENK- and VIP-immunoreactive nerve fibres within the muscle layer and myenteric plexus of the large intestine in a young female patient (aged 17 years) suffering from colitis ulcerosa activa *(CUA). As the CUA was found to be totally drug-resistant, a pancolotomy was performed by means of the Soave technique. Varicose nerve fibres, immunoreactive either to LENK or VIP, but not to both substances simultaneously, were found in all fragments of the bowel studied. A striking feature was their distribution pattern within the studied layers. In all cases LENK-IR fibres were closely accompanied by VIP-IR terminals. The density of the examined fibres depended on the bowel fragment studied, and was the greatest in the sigmoid colon, descending colon and rectum, while the lowest number was found in the caecum. The results of the present study may thus be indicative for the involvement of LENK- and VIP-IR nerve fibres in the control of bowel functions during CUA, possibly on the basis of a "cross-talk" between terminals running in close vicinity to each other.*

key words: colitis ulcerosa, enteric nervous system, plasticity, VIP, enkephalins, human

INTRODUCTION

The morphological basis of neuronal regulations within the large intestine, especially when affected by chronic disease, has not as yet been elucidated in detail. Previous studies have unequivocally suggested that neuropeptides (especially SP and VIP) may play an important role in the regulation of gastrointestinal functions during ulcerative colitis [3]. Pronounced changes have been observed in relation to the quantity and expression pattern of these substances in the inflamed colon, when compared to control tissues [4, 5]. Thus the aim of the present study was to disclose the detailed patterns of distribution and the interrelationships between VIP- (a relatively well-known agent that plays a crucial role in the formation, development and persistence of CUA in humans [3, 4]) and LENK-IR nerve fibres, (whose functions during CUA are still unknown) within the muscle layer and the myenteric plexus in the large intestine of a human subject suffering from CUA.

MATERIAL AND METHODS

The study was performed on cryostat sections from all fragments of the distal bowel resected from a female patient (aged 17 years) suffering from clinically diagnosed, drug-resistant CUA. The history of

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the illness was as follows: the first symptoms observed seven years ago, consisted of, on average, two exacerbations of the process per year, painful defecation with traces of blood but without body weight loss and signs of fever. Inflammatory changes in all fragments of the large intestine were observed colonoscopically during exacerbations, and histopathological examinations of biopsies revealed a typical picture of CUA. In the periods of remission, only weak inflammatory changes were observed in some fragments of the bowel. The patient was medicated by different drugs, including mesalazine, encorton, imuran, cyclosporin A, 6-mercaptopurin and metotrexate. However, as the CUA was totally drug-resistant, a pancolotomy was performed by means of the Soave method. Fragments from the caecum, ascendens, transverse, descendens and sigmoid colon, as well as from the rectum, were collected during the surgery and fixed by immersion in 4% paraformaldehyde. Ten-μm-thick cryostat sections were subjected to routine double immunofluorescence using combinations of antisera directed towards LENK and VIP. Secondary antisera were FITC- or biotin-conjugated and then visualised by the streptavidin-CY3 complex. Labelled sections were studied with an Olympus microscope equipped with epi-illuminator and appropriate filter sets for FITC and CY-3. The fibres within the muscle layer were counted in 12 randomly chosen fields and their number presented as a mean. The density of nerve terminals within the myenteric plexus was scored according to arbitrary units on a scale: from + (few fibres) to ++++ (a very dense mesh of nerve terminals).

RESULTS AND DISCUSSION

LENK- and VIP-IR nerve fibres were observed in all fragments of the large intestine studied. Moreover, an extremely close, characteristically morphological relationship between LENK- and VIP-IR fibres was observed within all the bowel segments studied (Fig. 1, 2). Although these fibres formed, in general, "pairs" of terminals running in very close proximity (Fig. 1), the co-localisation of the studied neuromediators in the same nerve fibre was never observed. However, the density of LENK- and VIP-IR terminals varied in the intestinal segments studied (Table 1). While the highest number of LENK-IR terminals was found within the sigmoid colon, descending colon and rectum, the lowest number was observed in the caecum (Table 1). The most numerous fibres containing VIP were located in the rectum, while the lowest number was found in the caecum (Table 1). The total density of labelled fibres (both LENK- and VIP-IR) was the highest within the rectum, whereas in the caecum they were the most sparsely distributed (Table 1). Similar numbers of fibres containing LENK or VIP were observed in the descending and transverse colon. In contrast to the rectum, the LENK-IR terminals were clearly more numerous than the VIP-IR fibres within the caecum and sigmoid colon. The results obtained may thus suggest that LENK- and VIP-IR fibres are involved in the fine control of colonic functions in humans during CUA, possibly on the basis of a "cross-talk" between terminals running in close vicinity to each other. This appears to be in agreement with previous studies implicating both LENK [1] and VIP [2] as factors that are involved in the neural control of the motility of the mammalian large bowel. However, while VIP is known as an important factor in colitis [3–5], it should be stressed that studies dealing with the function of LENK during this disease had not previously been performed. Therefore, although the role of enteric neuropeptides in CUA appears to be very important [3–6], their precise distribution pattern, as well as their importance in this disease, still remains controversial and requires further investigation.

Table 1. The density of LENK- and VIP-IR fibres in different parts of the large intestine studied (mean number of fibres *per* field studied)

Fragment studied	LENK-IR fibers	VIP-IR fibers
Caecum	9.1	3.2
Ascending and transverse colon	16.0	16.4
Descending colon	20.4	19.2
Sigmoid colon	25.3	19.4
Rectum	20.9	32.8

REFERENCES

1. Cupo A, Niel JP, Jule Y, Jarry T (1998) Enkephalins in the inferior mesenteric ganglion of the cat and in the area of the lower digestive tract innervated by this ganglion: quantification by radio-immunoassay and characterization by high pressure liquid chromatography. Neuropeptides, 12: 257–263.
2. Ekblad E (1999) Pharmacological evidence for both neuronal and smooth muscular PAC 1 receptors and a VIP-specific receptor in rat colon. Regul Pept, 84: 1–12.
3. Keranen U, Jarvinen H, Kiviluoto T, Kivilaakso E, Soinila S (1996) Substance P and vasoactive intestinal polypeptide-immunoreactive innervation in normal and in-

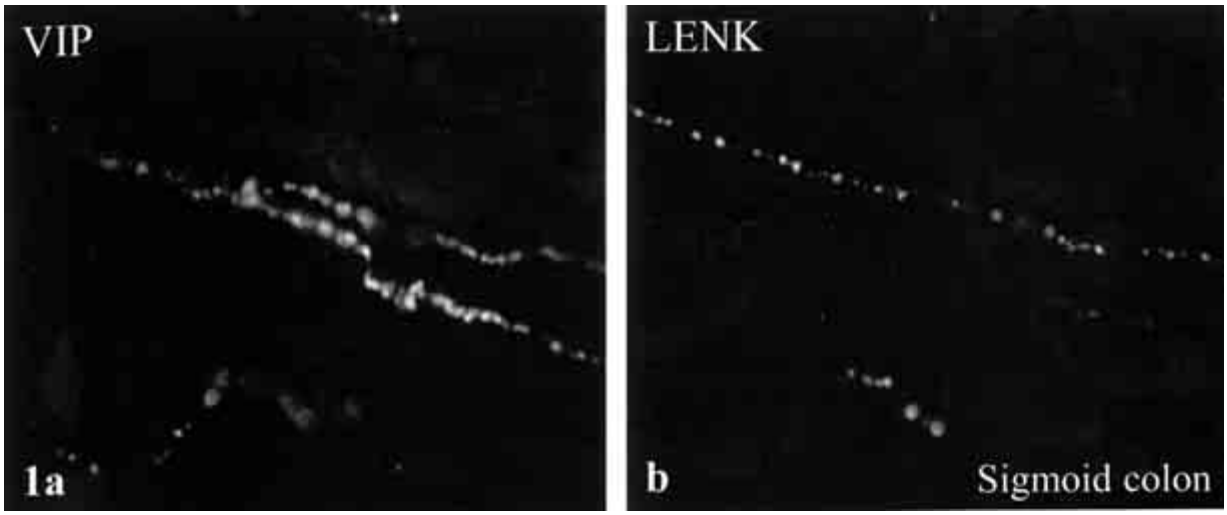

Figure 1. Double immunostaining. VIP- (**A**) and LENK-IR (**B**) varicose nerve fibres within the submucosa of the sigmoid colon. Such terminals were often observed to run in close proximity to one another; × 200.

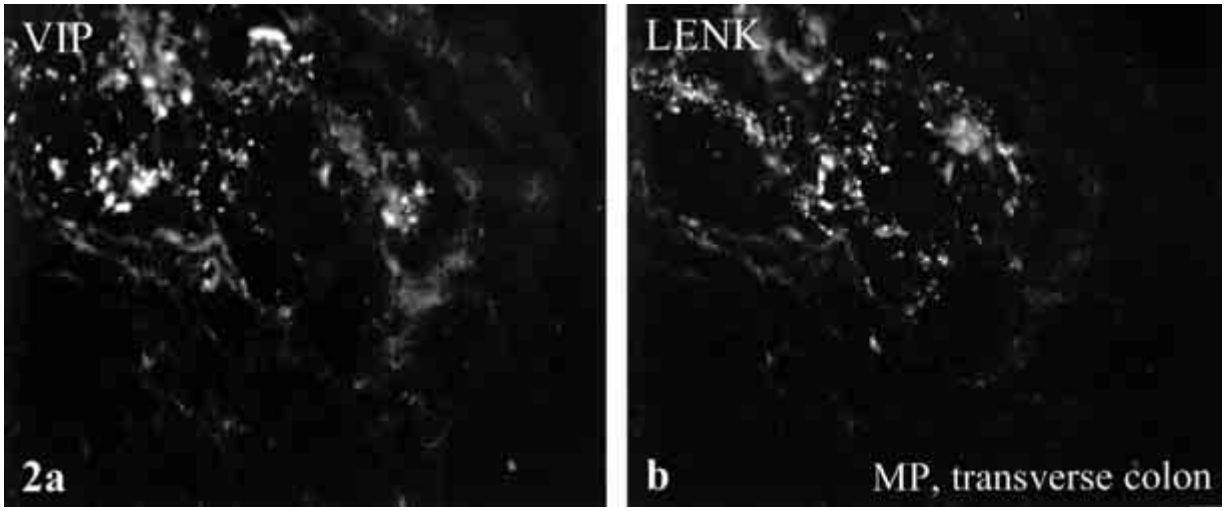

Figure 2. Double immunostaining. Dense mesh of VIP- (**A**) and LENK-IR (**B**) intraganglionic nerve terminals within the myenteric plexus of the transverse colon. Note the absence of terminals co-localising both substances; × 200.

flamed pouches after restorative proctocolectomy for ulcerative colitis. Dig Dis Sci, 41: 1658–1664.
4. Neulist M, Aubert P, Toquet C, Oreshkova T, Barouk J, Lehur PA, Schemann M, Galmiche JP (2003) Changes in chemical coding of myenteric neurones in ulcerative colitis. Gut, 52: 84–90.
5. Renzi D, Mantellini P, Calabro A, Panerai C, Amorosi A, Paladini I, Salvadori G, Garcea MR, Surrenti C (1998) Substance P and vasoactive intestinal polypeptide but not calcitonin gene-related peptide concentrations are reduced in patients with moderate and severe ulcerative colitis. Ital J Gastroenterol Hepatol, 30: 62–70.
6. Watanabe T, Kubota Y, Mutto T (1998) Substance P containing nerve fibers in ulcerative colitis. Int J Colorect Dis, 13: 61–67.