

**Aim of the study:** The purpose of this study was to investigate the presence of vasoactive intestinal polypeptide (VIP), neuropeptide Y (NPY) and calcitonin gene related peptide (CGRP) neuropeptides in neoplastic tissue of the large intestine. The results obtained were compared to an unchanged part of the intestine originating from the same patient.

**Material and methods:** The tissue samples were taken from the postoperative part of the intestine, resected during an operation due to treatment of colon cancer. Tissues were taken from 15 patients. Concentrations of neuropeptides were determined by ELISA tests, and results were calculated per 1 g of fresh tissue. The data obtained were statistically analysed.

**Results:** In tissues of the neoplastic tumour of the sigmoid colon and rectum the concentration of VIP and NPY was determined (28.9 ng/g and 8.6 ng/g, respectively). The observed values were statistically significantly lower than those observed in corresponding tissues of unchanged intestinal segments (83.9 ng/g for VIP and 19.5 ng/g for NPY). Surprisingly, amounts of CGRP were very similar in both neoplastic and intact tissues (13.3 ng/g and 13.4 ng/g respectively).

**Conclusions:** Immunoenzymatic investigations allow one to establish the concentration of neuropeptides associated with the enteric nervous system in cancer tissues of the large intestine. Various concentrations of these neuropeptides were observed in particular patients of both compared groups. The performed statistical analysis did not reveal associations between degree of cancer infiltration within the colonic wall and the level of presence of investigated neuropeptides in either cancer or control tissues. The fact of presence of these neuropeptides in cancer tissues confirmed by this study should be underlined at the same time.

**Key words:** colon cancer, enteric nervous system, neuropeptides, calcitonin gene related peptide, neuropeptide Y, vasoactive intestinal polypeptide.

# Concentration of vasoactive intestinal polypeptide, neuropeptide Y and calcitonin gene related peptide in neoplastic tissue of the human large intestine

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## Introduction

In the neurons of the enteric nervous system (ENS) besides classical neurotransmitters such as acetylcholine and noradrenalin there coexist other biologically active substances, including neuropeptides. In physiological conditions, within nervous structures supplying human large intestines the presence of vasoactive intestinal polypeptide (VIP), calcitonin gene related peptide (CGRP) and neuropeptide Y (NPY) was found [1-5].

Until now there have been no data regarding the presence of neuropeptides in neoplastic tissues of the human and animal large intestines. Studies dealing with neuropeptides in cancer tissues in organs other than the large intestine revealed the presence of these substances within organs studied. It was reported that VIP, CGRP and NPY were found in human non-malignant and malignant tissues of the prostate gland and urinary bladder [6]. Similarly, pituitary adenylyl cyclase-activating polypeptide (PACAP) is present in tumour tissues of the ovary, prostate gland and mammary gland [7-9]. In these papers except for confirmation of the presence of PACAP, VIP and NPY, there is no explanation of the possible role of neuropeptides mentioned above in carcinogenesis of these organs. On the other hand, other results lead to conclusions indicating neuropeptides as endogenous factors influencing cancer growth [10]. VIP is presumed to be such a factor influencing the growth of cancer cells of the lung, breast, prostate, colon and urinary bladder [11, 12]. The other studies dealing with the presence of neuropeptides in the cancer process showed significant increase of galanin in cancer tissue as well as in blood in a group of patients with colon cancer. This increase correlated with the degree of cancer invasion and stage of disease and could be considered as a novel colon cancer biomarker [13].

Taking into consideration the lack of data regarding the presence of neuropeptides in neoplastic tissue of the large intestine, we decided to investigate the concentration of VIP, NPY and CGRP in cancer tissues of the colon and rectum. The results obtained were compared to those from unchanged, control segments of the large intestine originating from the same patient.

## Material and methods

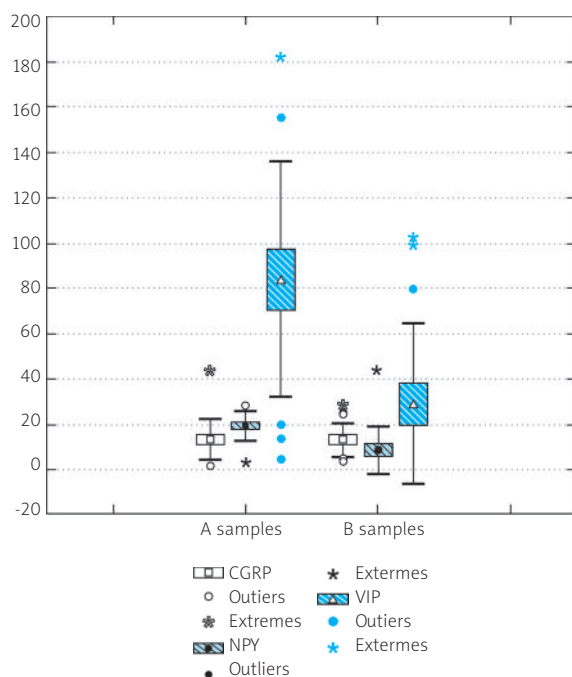
The tissues were taken from patients suffering from cancer of the sigmoid colon or rectum who underwent surgery at the Oncological Surgery Department in the Hospital of Warmia and Mazury Oncological Center between

13.07.2004 and 06.06.2005. Tissues were taken from 15 patients (9 females and 6 males). Average age of patients was 63.0; the youngest patient was 41, the oldest 78 years old (Permission of Bioethical Commission of the Regional Medical Board of Warmia and Mazury in Olsztyn No. 107/2004/II dated 01.06.2004).

Tissues were taken during resection of the sigmoid colon or anterior resection of the rectum, from patients who were in relatively good condition, without any other important diseases. Directly after resection of the intestinal segment, two small pieces comprising the entire wall of the intestine were cut out, one from the segment infiltrated by cancer, the second one from the part of the organ which showed no pathological changes, minimum 5-7 centimetres from the infiltrated part. Then, the samples were weighed and kept in liquid nitrogen until further processing. Tissue concentrations of neuropeptides were determined by ELISA tests (Table 1) (Peninsula Laboratories, INC) according to the instructions attached to the kit. Absorbance was measured with the immunoplate reader Dynex MRX (Dynex Technologies) with test filter 450 nm. A 10-point standard curve was prepared, and results were calculated per 1 g of fresh tissue. The data obtained were statistically analysed.

**Table 1.** ELISA kits (Peninsula Laboratories Inc.) used in this study

Substance	Cod	Lot
VIP	S-1183 (EIAH-7161)	021071
NPY	S-1220 (EIAH-7172)	021068
beta-CGRP	S-1200 (EIAH-6012)	021072



**Fig. 1.** Diagram showed an average quantity (in ng/g), standard error and standard deviation of each neuropeptide in A and B samples group within the wall of the large intestine

## Results

The data obtained showed a statistically significant decrease in the concentration of VIP in cancer tissues (B samples) as compared to control tissues (A samples) (28.94 ng/g and 83.87 ng/g of fresh tissue respectively). Calculated  $p$  level = 0.000058 did not exceed the assumed level of significance  $\alpha = 0.05$  (in the nonparametric Mann-Whitney test).

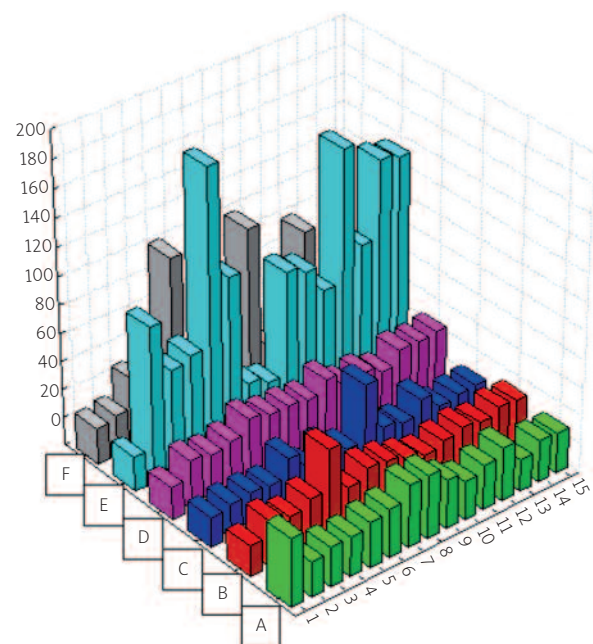
Statistically significant differences in NPY contents were also found between B and A samples. NPY concentration was lower in B samples (average is 8.67 ng/g) than that in A samples (average is 19.49 ng/g);  $p = 0.000001$ , not exceeding assumed level of significance  $\alpha = 0.05$ .

The results of examinations show similar contents of CGRP in B and A samples (13.33 ng/g and 13.35 ng/g of fresh tissue respectively). Calculated probability  $p = 0.604839$  exceeded the level of significance  $\alpha = 0.05$ .

The statistical analysis did not reveal correlations between degree of cancer invasion within the colonic wall and the concentrations of investigated neuropeptides in either cancer or control tissues.

## Discussion

Previous studies have shown the high presence of VIP in the wall of the small and large intestines in humans. Higher content of VIP was observed in the muscular layer in comparison to the mucosal and submucosal membrane. It was also established that VIP-ergic components of the ENS induce relaxation of the muscular membrane of the intestines, increase intestinal secretion and relax the intesti-



**Fig. 2.** Differences of neuropeptides concentrations in particular patients in both compared groups. 1-15 numbers of patients; A – CGRP in A samples, B – CGRP in B samples, C – NPY in A samples, D – NPY in B samples, E – VIP in A samples, F – VIP in B samples

**Table 2.** Concentration of investigated neuropeptides in large intestine, results of the ELISA tests, (A – samples taken from part of intestine without pathological changes, B – samples taken from cancer infiltration)

	Average quantity of neuropeptide: ng/g of fresh tissue	Standard deviation: ng/g of fresh tissue	Minimum quantity of neuropeptide: ng/g of fresh tissue	Maximum quantity of neuropeptide: ng/g of fresh tissue
VIP – A samples	83.87	55.51	4.35	188.07
VIP – B samples	28.94	35.11	4.03	110.08
NPY – A samples	19.49	6.69	2.57	30.95
NPY – B samples	8.67	11.60	1.19	62.69
CGRP – A samples	13.35	9.04	0.46	46.82
CGRP – B samples	13.33	8.34	2.44	36.04

nal blood vessels [1, 14-22]. The study reports present the tissue concentration of VIP, NPY and CGRP in the large intestinal wall infiltrated by neoplasm. A statistically significant decrease in the level of VIP in the intestinal wall infiltrated by neoplasm was found as compared to intact tissue. Similar changes were observed in patients with pathological constipation [23-25]. This related result may confirm impaired peristalsis due to colon cancer invasion. The other study showed no changes in VIP tissue concentration in large intestine samples collected from pigs suffering from inflammation provoked by the bacteria *Brachyspira hyodysenteriae* [26]. Such differences can be explained by the difference between inflammatory and neoplastic processes. In the above case the inflammatory process was acute and included a large part of the intestine or even the whole intestine. It is also possible that the mechanism of reaction of the nervous system in neoplasia is different.

In the human large intestine the presence of NPY was found mostly in the muscular layer, and also a large amount of NPY was found within the sphincters of the gastrointestinal tract of man, which may suggest a role of this neuropeptide in the regulation of the tonus of the musculature of these anatomical structures. It was also found that NPY inhibits the contraction of the intestinal muscular layer [2, 17, 19, 27, 28]. The present study showed lower concentration of NPY in tissues of the large intestine infiltrated by cancer than in healthy intestinal tissues. In other studies, no changes were observed in the level of NPY within the wall of the large intestines in the course of such diseases as diverticular disease of the large intestines and idiopathic constipation as well as postradial enteropathy [23, 24]. In the experimental studies performed on animals concerning plasticity of innervation of the gut in the acute inflammatory process, it was found that the concentration of NPY in the wall of the large intestine in the pig increased [26].

It could be accepted correlations between decrease of NPY concentrations in cancer tissue with lower density of the NPY-ergic nerve fibres and perikarya observed in previously conducted microscopic examinations. Confirmed changes of NPY presence may suggest disturbed proper colon peristalsis.

CGRP is present in sensory and excretory compartments of the ENS. Additionally, immunoreactivity to CGRP was

found in endocrine cells in the human small intestine [5, 19, 21]. In the present study, CGRP was found in tissues of the large intestine obtained from patients suffering from intestinal cancer. The levels of CGRP were similar in both neoplastic and control tissue samples, but it should be stressed that concentration of this neuropeptide differed between the particular samples (decreases and increases were observed). In 8 of 15 studied samples, an increase in CGRP concentration was observed. Such a result can be explained by the presence of CGRP not only in neuronal elements of the intestine but also in endocrine cells of the mucosa, which was previously described in humans by Timmermans [5]. It should also be stressed that solid tumour infiltration constituted a large portion of the sample and the mucosa within the cancer tissues was destroyed. Localization of CGRP in both above-mentioned sites of the intestines undoubtedly hampered the correct interpretation of the results obtained in this study. This interpretation is not helped by the fact that in neoplasm infiltrated tissues of the genitourinary tract (urinary bladder, prostate) other scientists found a significant decrease of CGRP concentration [6]. The surprisingly high level of CGRP determined in the present study (more than half of "pathological" samples) requires further detailed investigations. Other investigations carried out on experimental animals are not very helpful to interpret these results. In experimentally evoked acute inflammation of the large intestine in laboratory animals, at the beginning of the inflammatory process, a decrease in CGRP content in all layers of the intestinal wall was observed, but then a gradual increase in the peptide concentration was observed. It should be underlined that CGRP is inherent in sensory neurons of ENS [29-33].

This study has confirmed the presence of VIP, NPY and CGRP within neoplastic tissues of the human large intestine. It could be assumed that these substances are localized within persistent neuronal elements in cancer infiltration. Unfortunately, this assumption was not confirmed by immunohistochemical investigations, which revealed absence of neuronal elements within solid cancer tissues. However, malformation of the nervous structures in the close vicinity of the cancer infiltration was found [34]. And finally, certainly surprising are the results dealing with CGRP concentrations which were similar in both pathological and control tissue samples. The only possible explanation of this

finding is that in neuronal elements displaced by cancer infiltration there is a particularly high concentration of this neuropeptide, significantly higher than in the rest of the intestine wall. Considering this fact, higher concentration of CGRP in components of ENS destroyed by cancer could be a focus of further investigation of this neuropeptide as a colon cancer biomarker, similar to galanin mentioned in the introduction.

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