

**CAPSAICIN-SENSITIVE AFFERENTATION AND HUMAN  
GASTROINTESTINAL TRACT**

Doctoral (Ph.D) Dissertation

András Dömötör MD

Science of Pharmacology Doctoral School

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First Department of Medicine, Medical and Health Centre, University of  
Pécs, Hungary



Program leader: Professor Lajos Botz, PhD, ScD

Supervisor: Gyula Mózsik, MD, PhD, ScD, Professor emeritus

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Abbreviations .....	4
<b>1. Introduction.....</b>	<b>5</b>
<b>2. “Immunohistochemical distribution of vanilloid receptor, calcitonin-gene-related peptide and substance P in gastrointestinal mucosa of patients with different gastrointestinal disorders” .....</b>	<b>10</b>
2.1. Introduction.....	10
2.2. Aim .....	11
2.3. Materials and methods.....	12
2.4. Results.....	13
2.5. Discussion and conclusion.....	22
<b>3. “Participation of capsaicin-sensitive afferent nerves in the gastric mucosa of patients with <i>Helicobacter pylori</i> positive or negative chronic gastritis” .....</b>	<b>24</b>
3.1. Introduction.....	24
3.2. Aim .....	25
3.3. Materials and methods.....	25
3.4. Results.....	27
3.5. Discussion and conclusion.....	32
<b>4. "Capsaicin-sensitive afferentation represents an indifferent defensive pathway from eradication in patients with <i>Helicobacter pylori</i> gastritis" .....</b>	<b>34</b>
4.1. Introduction.....	34
4.2. Aim .....	35
4.3. Materials and methods.....	35
4.4. Results.....	37
4.5. Discussion and conclusion.....	39
<b>5. “Capsaicin and glucose absorption and utilization in healthy human subjects” 42</b>	<b>42</b>
5.1. Introduction.....	42
5.2. Aim .....	43
5.3. Materials and methods.....	43
5.4. Results.....	44
5.5. Discussion.....	45
<b>6. Brief general conclusion and near (present) future .....</b>	<b>47</b>
<b>7. Acknowledgements .....</b>	<b>52</b>
<b>8. References .....</b>	<b>53</b>

<b>9. Publication related to the dissertation .....</b>	<b>61</b>
<i>Published papers.....</i>	<i>61</i>
<i>Book Chapters.....</i>	<i>62</i>
<i>Book .....</i>	<i>62</i>
<b>10. Publications not related to the dissertation .....</b>	<b>63</b>
<i>Oral presentations and posters.....</i>	<i>63</i>
<i>Abstracts .....</i>	<i>65</i>

## Abbreviations

<b>ASA</b>	acetylsalicylic acid
<b>BAO</b>	basal acid output
<b>CGRP</b>	calcitonin gene-related peptide
<b>d</b>	day
<b>ED<sub>50</sub></b>	50 % effective dose
<b>GAL</b>	galanin
<b>GI</b>	gastrointestinal
<b>GTPD</b>	gastric transmucosal potential difference
<b>H</b>	hour
<b>H<sub>2</sub>R</b>	histamine receptor type 2
<b>H. pylori</b>	Helicobacter pylori
<b>IND</b>	indomethacin
<b>i.g.</b>	intragastrically
<b>i.v.</b>	intravenous
<b>MALT</b>	mucosa-associated lymphoid tissue
<b>min</b>	minute
<b>NPY</b>	neuropeptide Y
<b>NSAID</b>	Nonsteroidal anti-inflammatory drug
<b>pA<sub>2</sub></b>	the negative logarithm of K(A), which is the concentration at which the 50% of the receptors are bound
<b>pD<sub>2</sub></b>	the negative logarithm of ED <sub>50</sub>
<b>PPI</b>	proton pump inhibitor
<b>s.c.</b>	subcutaneous
<b>SP</b>	substance P
<b>SS</b>	somatostatin
<b>TRPV1</b>	transient receptor potential vanilloid 1
<b>VIP</b>	vasoactive intestinal peptide
<b>[<sup>13</sup>C] UBT</b>	[ <sup>13</sup> C] urea breath test

## 1. Introduction

Capsaicinoids are the active ingredients of red pepper and paprika. These plants have been well known in about 7000 years, and these have been applying in the every day of the culinary practice.

An important discovery was made by Jancsó et al. (1, 2, 3) that capsaicin (capsaicin, dihydrocapsaicin, nordihydrocapsaicin and other capsaicinoids) could specifically modify the function of capsaicin-sensitive afferent nerves. The actions of capsaicin on the capsaicin-sensitive afferent nerves has been found to be dose-dependent (4, 5, 6, 7, 8,9, 10, 11).

Szolcsányi (6) observed four different stages of capsaicin action on afferent nerves (depending on the dose and duration of the exposure of the compound), which include: (a) excitation (stage 1); (b) sensory blocking effect (stage 2); (c) long-term selective neurotoxin impairment (stage 3); and (d) irreversible cell destruction (stage 4). The stages 1 and 2 are reversible, whereas stages 3 and 4 are irreversible compound-induced actions on the capsaicin-sensitive afferent nerve. These stages of capsaicin actions can be detected in the gastrointestinal tract (11).

Capsaicin activates capsaicin (vanilloid) receptors expressed by a subgroup of primary afferent nociceptive neurons (8). The capsaicin receptor has been cloned (12) and has been found to be linked to a cation channel. It is gated by capsaicin and other capsaicinoids (some vanilloids) by various treatments including low pH, noxious heat and various pain-producing endogenous and exogenous chemicals. Thus, those sensory nerve endings possessing these ion channels are susceptible to being stimulated in the gastric mucosa. During administration of small doses of capsaicin (from ng/kg to µg/kg body weight) neurotransmitters [substance P (SP), calcitonin-gene related peptide (CGRP), somatostatin (SS), etc.,] are released from this nerve endings and these mediators are responsible for the physiological effects of capsaicin in the gastrointestinal (GI) tract (8, 13, 14).

The vagal nerve has a key-role in the development of gastrointestinal mucosal damage and prevention (15). The key-role of the vagal nerve has been emphasized dominantly in the aggressive processes to gastrointestinal mucosa (such as in peptic ulcer disease, gastric mucosal damage, etc.) as evidenced from gastrointestinal investigations in animal models and as well as in human clinical practice. Thus, “chemical” and

“surgical” vagotomy was widely used in the treatment of patients with peptic ulcer disease over the years up to mid-1970s (16). The primary aims of this therapy were then to decrease the activity of vagal nerve at the level of efferent vagal fibers. The application of capsaicin in the animal experiments was used as a specific tool to investigate those primary afferent nociceptive neurons (8, 13, 14, 17, 18, 19) involved in the different physiological and pathological processes.

Szolcsányi and Barthó (5) were the first authors, who clearly identified the beneficial and harmful effect of capsaicin in experimental peptic ulcer in rats, following varying doses of capsaicin. Later, Holzer undertook extensive investigations on the mode of action of capsaicin on gastrointestinal functions (13, 14, 17, 18). Our work team also contributed during the 1980's to gastrointestinal capsaicin research from studies in animal models (19).

Recently the new drug, lafutidine was introduced in the medical treatment of gastrointestinal mucosal damage (20, 21, 22, 23, 24). Lafutidine is a histamine receptor type 2 (H<sub>2</sub>R) blocking compound which uniquely has typical capsaicin actions on the target organ.

The new and interesting results obtained with capsaicin application in animal experiments offered an excellent tool to approach the different events of human gastrointestinal physiology, pathology and pharmacology.

The clinical studies with capsaicin have been started from 1997 by our work team and the milestones of these observations are the followings:

- **Determination of gastric basal acid output (BAO) in normal human healthy subjects.**

Capsaicin at doses of 100, 200, 400 and 800 µg orally dose-dependently inhibited the gastric acid output. The 50 % effective dose (ED<sub>50</sub>) of capsaicin on the inhibition of BAO was 400 µg/person. The dose-response curves were then constructed (25, 26).

- **Determination of affinity and intrinsic activity curves for drugs inhibiting the gastric BAO in healthy human subjects.**

There was no competitive effects of drugs [capsaicin (100, 200, 400 and 800 µg intragastrically[i.g.]), atropine (0.1–1.0 mg subcutaneous [s. c.]), pirenzepine (25–50 mg), famotidine (20–40 mg orally), ranitidine (150–300 mg orally), cimetidine (100–1000 mg orally), omeprazole (20–40 mg intravenous [i. v.]) or

esomeprazole (20–40 mg orally)] exist on the gastric BAO. From investigations of the molecular pharmacological action of drugs, the negative logarithm of  $ED_{50}$  ( $pD_2$  -the most frequently used value for characterization of the potency of a drug) values and the intrinsic activity (compared with the effects of atropine) and the negative logarithm of  $K(A)$  ( $pA_2$ ) values clearly show that capsaicin acts at lower doses compared with those drugs acting on the muscarinic (atropine, pirenzepine) or  $H_2R$  (cimetidine, ranitidine, famotidine) and on proton pump (omeprazole, esomeprazole) (27).

- **Calculation of “parietal” and “non-parietal” components of gastric secretory responses with and without capsaicin.**

The measurements of changes in the concentrations of cations ( $H^+$ ,  $Na^+$ ,  $K^+$ ,  $Ca^{2+}$ ,  $Mg^{2+}$ ) and  $Cl^-$  enable identification of the “parietal” and “non-parietal” components of gastric secretion in humans (Hollander’s method) without and with administration of different drugs (or compounds) (28). Using this method for the calculation and evaluation of measurements of cations, chloride in the gastric juice indicated clearly the significant decrease of “parietal component” ( $\Delta - 18 \pm 2$  mmol/L,  $P < 0.001$ ) in association with significant increase of “non-parietal” ( $\Delta + 19 \pm 2$  mmol/L,  $P < 0.001$ ) component of the gastric secretion after administration of 400  $\mu g$  (given orally) capsaicin ( $n = 10$ ) (26). The albumin concentration increased from  $1.24 \pm 0.001$  g/L vs.  $1.63 \pm 0.02$  g/L ( $P < 0.001$ ;  $n = 10$ ) after capsaicin (400  $\mu g$  i. g. given) application (26).

- **Measurement of the changes in the gastric transmucosal potential difference (GTPD) induced by capsaicin in the healthy human subjects.**

Capsaicin (given i. g. in doses of 100, 200, 400 and 800  $\mu g$ ) dose-dependently increased the GTPD alone [ $\Delta$  value from to baseline 10 ( $-mV$ )]. When administered at twice the same dose of capsaicin (800  $\mu g$ , i. g.) there was a similar increase in GTPD over the same time period (26, 29).

- **Demonstration of the capsaicin’s effect on ethanol-induced changes of GTPD.**

Intragastrically administered ethanol caused an immediate significant decrease in the GTPD [ $\Delta 25$  ( $-mV$ )]. The i.g. applied capsaicin (given in doses of 100, 200, 400 and 800  $\mu g$ ) dose-dependently prevented the ethanol-induced decrease of GTPD in human healthy subjects (26, 30).

- **Measurement of gastric microbleeding produced by acute administration of indomethacin (IND) (without or with simultaneously administration of capsaicin) in healthy human subjects.**

The baseline of blood loss was  $2.0 \pm 0.2$  mL/day (d) (n = 14) while that following administration of IND, which was increased to  $8.1 \pm 0.2$  mL/d (n = 14;  $P < 0.001$ ). Capsaicin (given in doses of 200, 400 and 800  $\mu$ g orally before the administration of IND) prevented in a dose-dependent manner the IND induced increased gastric microbleeding in normal healthy human subjects (26, 31).

- **Measurement of gastric emptying by stable isotope method. Measurements of gastric emptying were performed on two consecutive days with the same protocol, without (first day) and with (second day) capsaicin.**

Capsaicin given i.g. at ED<sub>50</sub> increased significantly the gastric acid emptying (32, 33).

The role of the capsaicin-sensitive afferent nerves are demonstrated by the further observation of our work team in *healthy human subjects* but not in *patients suffered from different gastrointestinal disorders*.

The answers of the following questions could help to understand the potential effects of capsaicin and the role of the capsaicin-sensitive nerves in human gastrointestinal disorders:

1. Do the capsaicin-sensitive afferent nerves take part in the development of different disorders in patients?
2. What kind(s) of mechanism(s) is (are) expected in the capsaicin-sensitive afferent nerves?
3. Do the capsaicin-sensitive afferent nerves (transient receptor potential vanilloid 1 [TRPV1]) and the liberated neurotransmitters (CGRP and SP) show a characteristic immunomorphology in different human GI disorders?
4. Is there any similarity in the gastric and large bowel disorders with respect to immunohistochemical distribution of TRPV1, CGRP and SP?
5. Is there any role of the capsaicin-sensitive afferent nerves (TRPV1) and the liberated neurotransmitters (CGRP and SP) in the development of chronic gastritis (as a pathomorphological appearance of inflammation in the gastric mucosa caused by different agents)?



6. Is there any difference in the immunostainings of TRPV1, CGRP and SP between the *Helicobacter pylori* (*H. pylori*) positive or negative chronic gastritis?
7. Are there any changes in the immunohistochemical distribution of TRVP1, CGRP and SP in the gastric mucosa of the patients with chronic gastritis produced by *H. pylori* before and after eradication therapy?
8. Have the capsaicin sensitive afferent nerves other (not only mucosal) effect in human healthy subjects?

The aim of this dissertation is to summarize the research that shows the possibility of the use of capsaicin (as a new research tool), to investigate its different aspects in human pathology and pharmacology.

## **2. “Immunohistochemical distribution of vanilloid receptor, calcitonin-gene-related peptide and substance P in gastrointestinal mucosa of patients with different gastrointestinal disorders”**

### **2.1. Introduction**

The principal role(s) of efferent vagal nerves has (have) been emphasized in the development of gastrointestinal mucosal damage and prevention, as well as in medical treatment (anticholinergic agents, histamine H<sub>2</sub>R inhibitors, proton pump inhibitors [PPI's]) in the last century.

From the initial observation of the capsaicin desensitization phenomenon, a long-lasting chemoanalgesia and impairments in thermoregulation against heat in the 1970s, the chain of new discoveries which led to the discovery of the capsaicin receptor on C-polymodal nociceptors has been briefly summarized elsewhere (8).

Neurogenic inflammation is mediated by these C-afferents which are supplied by the putative capsaicin receptor. These afferents are called capsaicin-sensitive chemoreceptive afferents. They opened new avenues in local peptidergic regulation in peripheral tissues. It has been suggested that, in contrast to the classical axon theory, the capsaicin-sensitive sensory system has a dual sensory afferent function whereby initiation of afferent signals and neuropeptide release are coupled at the same nerve endings. Furthermore, in the skin at threshold stimuli which do not evoke sensation already maximum efferent response as enhanced microcirculation is elicited. Recently the capsaicin receptor has been cloned and it is now named TRPV1 (12). Hence, capsaicin research led to discovery of the first temperature-gated ion channel gated by noxious heat, protons, vanilloids and endogenous ligands as anadamide, N-oleodopamine and lipoxygenase products. Another recent achievement is the discovery of a novel, unorthodox neurohormonal regulatory mechanism mediated by somatostatin. SS released from the TRPV1-expressed nerve endings reaches the circulation and elicits systemic anti-inflammatory and analgetic sensory functions.

The possible role of afferent vagal nerve was studied in the last decades both in development of gastrointestinal mucosal and protection in animal experiments (9, 14, 19, 34). Recently the gastroprotective effect of capsaicin against chemical agents (ethanol, IND) has been proven in human healthy subjects (26, 33). The beneficial

effect of capsaicin was also proven in patients with functional gastrointestinal disorders (35, 36).

In the vagal nerves, only 10% is constituted by efferent nerve fibers and 90% is constituted by afferents ones; however, only 9% of the afferent nerves represents the capsaicin-sensitive afferent nerves (37, 38).

Only a few papers can be found suggesting that the functional influence between the efferent and afferent nerve fibers of the vagus under different experimental circumstances (19).

The overlap between the efferent and capsaicin-sensitive afferent vagal nerve was proven during the omeprazole effect due to gastric acid secretion and IND induced gastric mucosal protection (39).

We have no scientific information on the involvement of capsaicin-sensitive vagal nerves in the development and prevention of different gastrointestinal diseases in patients. In our present study we demonstrated the presence of capsaicin/vanilloid receptor (TRPV1) and the released neuropeptides (CGRP and SP) in capsaicin-sensitive afferent nerves. Together, these specific immunohistochemical observations demonstrate the involvement of capsaicin-sensitive afferent nerves in the human GI disorders.

## **2.2. Aim**

The aims of this study were: (1) collect patients with different gastric (gastritis, erosive gastritis, gastric ulcer, polyps, adenocarcinoma) and large bowel (chronic inflammatory bowel diseases, colon polyps without and with hyperplasia, dysplasia, adenocarcinoma) diseases; (2) demonstrate the immunohistochemical participation and distribution of TRPV1, CGRP and SP in these patients; (3) answer the following questions: (i) Do the capsaicin-sensitive afferent nerves take part in the development of different disorders in patients? (ii) What kind(s) of mechanisms is (are) expected in the capsaicin-sensitive afferent nerves? (iii) Do the neurotransmitters show a characteristic immunomorphology? (iv) Is there any similarity in the gastric and large bowel disorders with respect to immunohistochemical distribution of TRPV1, CGRP and SP?

### **2.3. Materials and methods**

The patients were admitted in the First Department of Medicine, Medical and Health Centre, Pécs University for medical (physical, laboratory, ultrasonography, endoscopic and histological) examinations. Patients (127 in total) suffering different gastric (gastritis, gastric ulcer, polyps, adenocarcinoma) and colon (chronic inflammatory diseases, polyps without and with hyperplasia, adenocarcinoma) diseases were included into this study. Thirty people with functional dyspepsia were taken as healthy controls. These patients and persons with functional dyspepsia (as controls) underwent the classical medical physical examinations and thereafter laboratory, ultrasonography, endoscopic examinations. For the control group, the results of the examinations were all negative. The age of patients was 21–84 years, the patient group consisted of 68 males and 59 females, and the functional dyspepsia group consisted of 10 males and 10 females. The patients had superficial gastritis (51), erosive gastritis (5), gastric ulcer (4), gastric polyps (6), gastric adenocarcinoma (4), inflammatory bowel disease (17), polyps and hyperplasia (17), polyps with dysplasia (17) and colon adenocarcinoma (6).

Biopsies were taken from all patients and control persons, and were histologically analyzed in the Department of Pathology. The patients were put into the different groups according to classical pathological diagnosis.

Immunohistological studies were carried out on paraffin-embedded tissue samples using the peroxidase-labeled polymer method (Lab Vision, USA). SP was detected by the NC1/34 HL rat monoclonal antibody, the TRPV1 and CGRP were labeled using polyclonal rabbit antisera (all from Abcam, UK).

Immunohistochemical analysis was assessed by light microscopy (Olympus). TRPV1 and CGRP staining was scored as positive or negative, SP as weak, medium or strong.

## 2.4. Results

The results are present in typical pictures in the different histologically classified groups of patients.

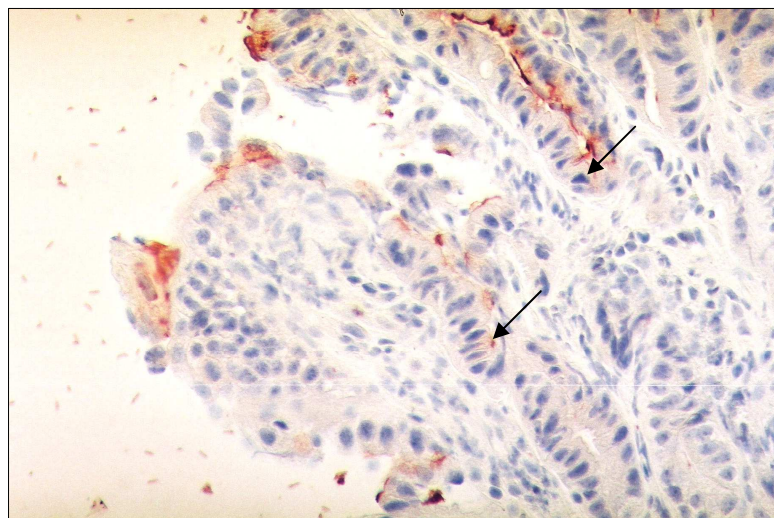
### 2. 4.1. Patients with gastric disorders

#### 2. 4.1.1. TRPV1

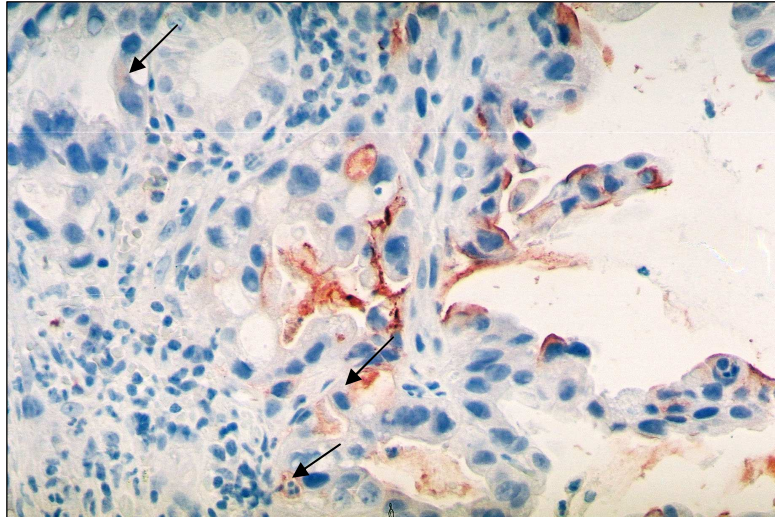
The TRPV1 was detected in chronic gastritis (Fig. 2.1), erosive gastritis (Fig. 2.2) and adenocarcinoma (Fig. 2.3). The results of TRPV1 evaluation in gastric tissues of patients with different gastric diseases are summarized in Fig. 2.4.



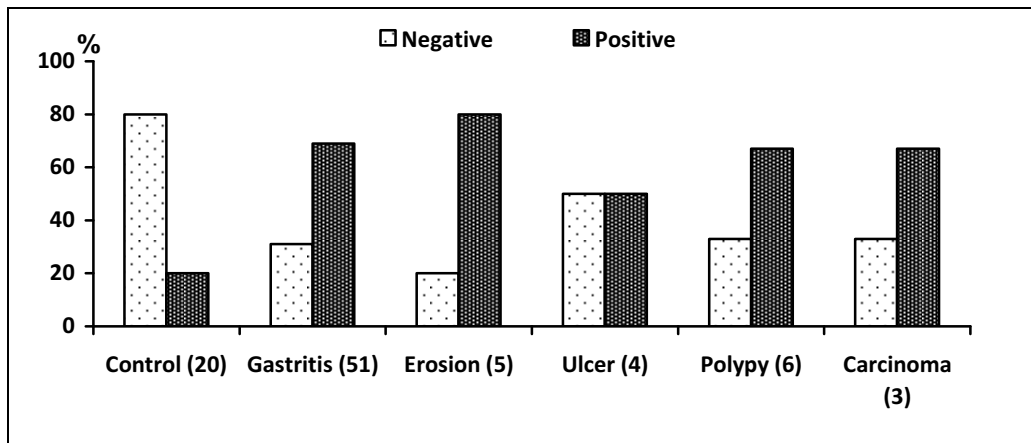
**Figure 2.1.** Demonstration of TRPV1 in the gastric mucosa of patients with chronic gastritis. Arrows show the immunsigns in the gastric mucosa.



**Figure 2.2.** Demonstration of TRPV1 in patients with erosive gastritis. Arrows show the immunsigns in the gastric mucosa.



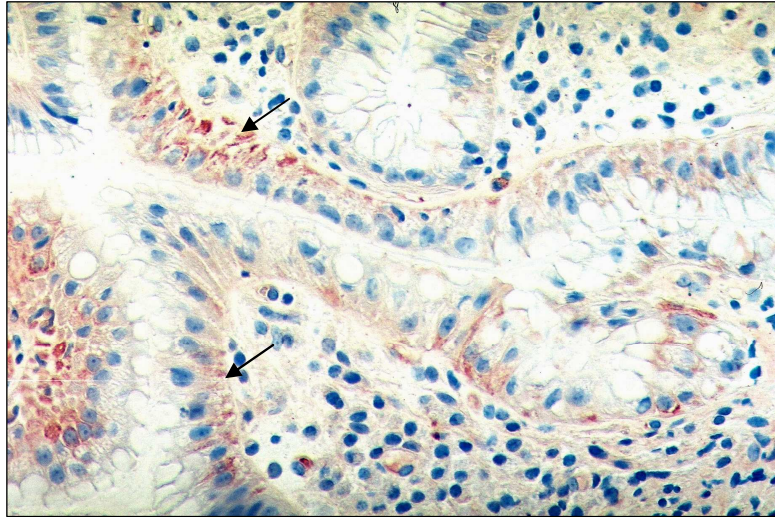
**Figure 2.3.** Demonstration of TRPV1 in the gastric mucosa of a patient with gastric adenocarcinoma. Arrows show the immunsigns in the gastric mucosa.



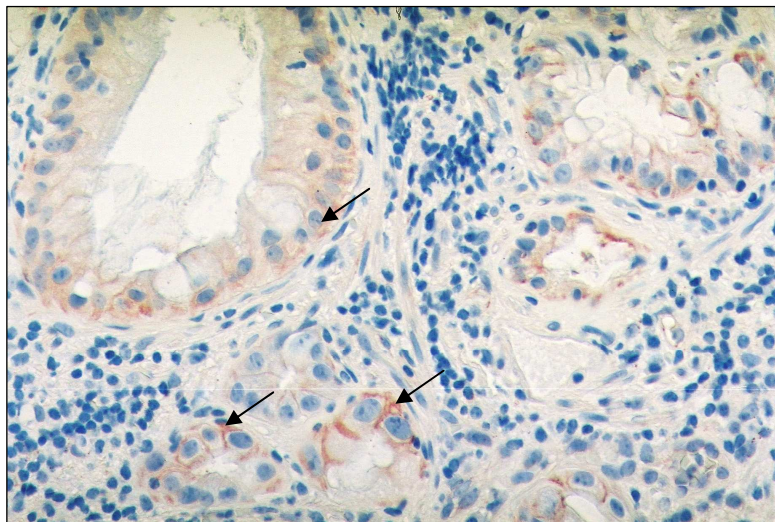
**Figure 2.4.** Summary of distribution of positivity and negativity of TRPV1 in gastric mucosa of patients with different gastric disorders. The numbers between the parentheses represent the number of the patients.

#### 2. 4.1.2. CGRP detection

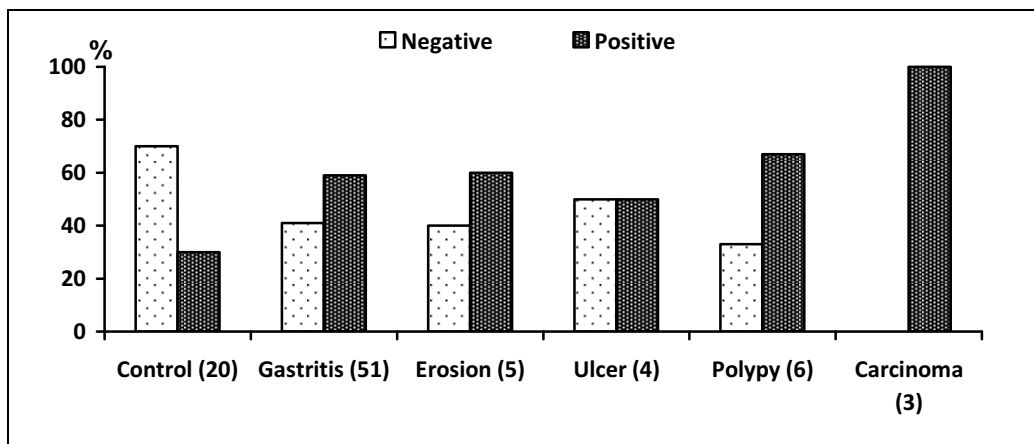
CGRP was detected in chronic gastritis (Fig. 2.5), and gastric polyps with severe dysplasia (Fig. 2.6). The results of CGRP evaluation in the gastric tissues of patients with different gastric diseases are summarized in Fig. 2.7.



**Figure 2.5.** Demonstration of CGRP in the gastric mucosa of a patient with gastric erosion. Arrows show the immunsigns in the gastric mucosa.



**Figure 2.6.** Demonstration of CGRP in the gastric mucosa of a patient with serve dysplastic gastric polyp. Arrows show the immunsigns in the mucosa.

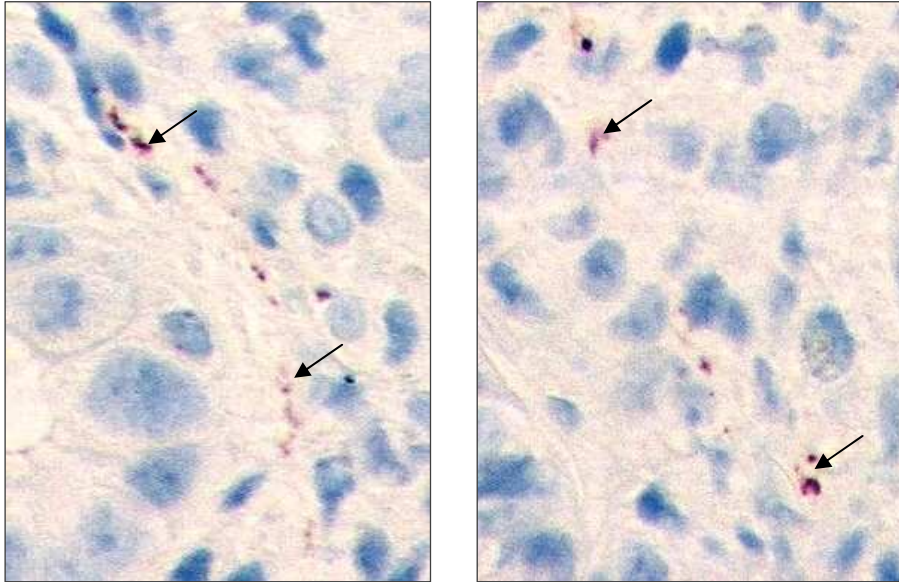


**Figure 2.7.** Summary of distribution of positivity and negativity of CGRP in gastric mucosa of patients with different gastric disorders. The numbers between the parentheses represent the number of the patients.

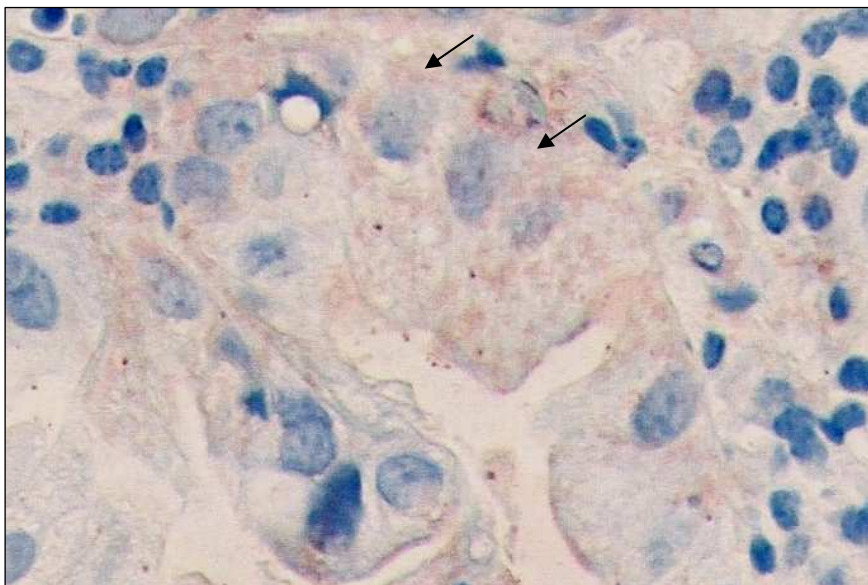
#### 2.4.1.3. SP detection

SP was detected in the gastric mucosa of the patient with chronic gastritis (Fig. 2.8) and in patients with adenocarcinoma (Fig. 2.9) but the immunosigns were not the similar.

The results of SP detection in the different gastric disorders are summarized in Fig. 2.10.

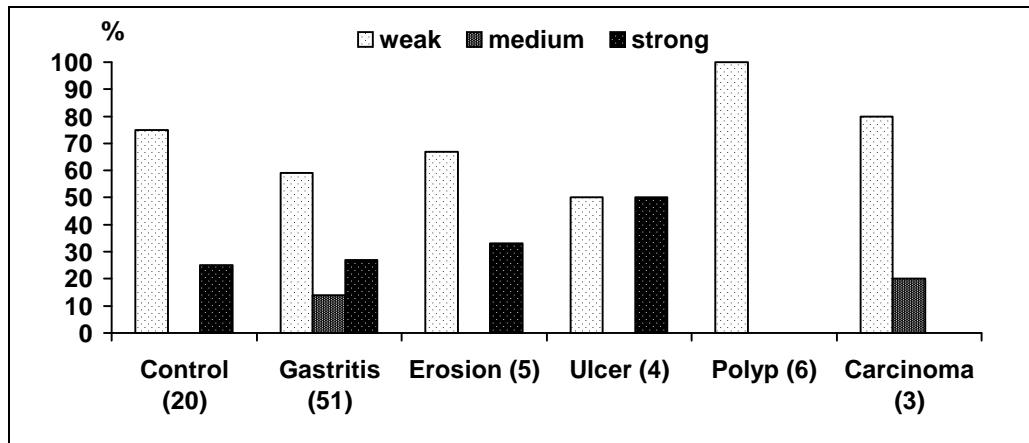


**Figure 2.8.** Demonstration of SP in the gastric mucosa of a patient with chronic gastritis. Arrows show the immunosigns in the mucosa.



**Figure 2.9.** "Smoke-like" intracellular positivity of SP in the gastric mucosa of a patient with gastric adenocarcinoma. Arrows show the immunosigns in the mucosa.





**Figure 2.10.** Summary of SP distribution in gastric mucosa of patients with different gastric disorders. The numbers between the parentheses represent the number of the patients.

Table 2.1. summarizes the immunohistochemical changes of TRPV1, CGRP and SP in biopsies from patients with different gastric diseases (gastritis, erosive gastritis, ulcer, polyps and carcinoma).

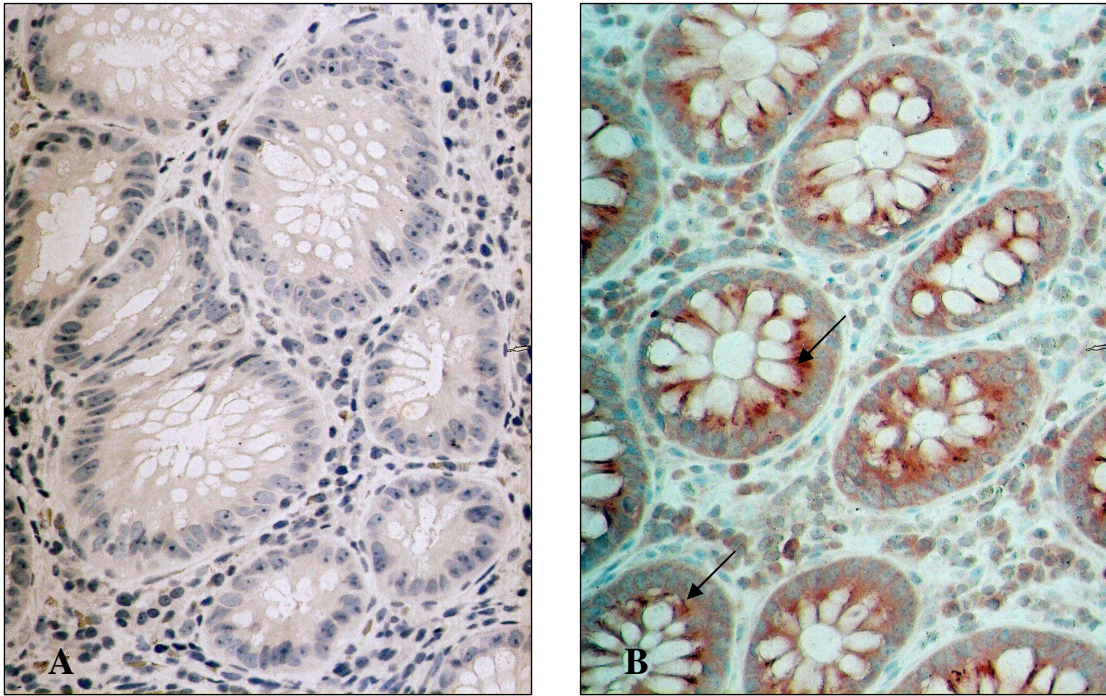
**Table 2.1.** Summary of immunohistochemical changes in the gastric mucosa in patients with different gastric disorders. The numbers between the parentheses represent the number of the patients.

	Control	Chronic gastritis	Gastric erosion	Peptic ulcer	Gastric polyps	Adenoc. ventriculi
<b>TRPV1</b>						
positive	20% (4)	69% (35)	80% (4)	50% (2)	66.7% (4)	66.7% (2)
negative	80% (16)	31% (16)	20% (1)	50% (2)	33.3% (2)	33.3% (1)
<b>CGRP</b>						
positive	30% (6)	59% (30)	60% (3)	50% (2)	66.7% (4)	100% (4)
negative	70% (14)	41% (21)	40% (2)	50% (2)	33.3% (2)	0% (0)
<b>SP</b>						
weak	75% (15)	59% (29)	66.7 % (4)	50% (2)	100% (6)	80% (3)
moderate	0% (0)	14% (7)	0% (0)	0% (0)	0% (0)	20% (1)
strong	25% (5)	27% (13)	33.3% (2)	50% (2)	0% (0)	0% (0)

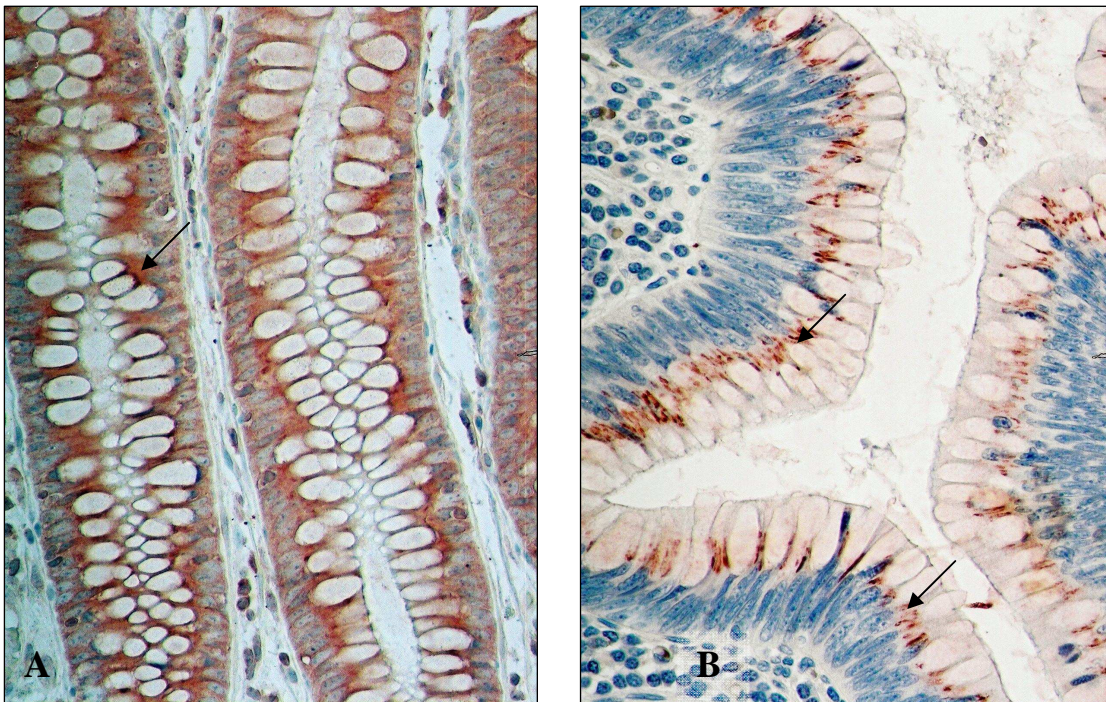
## 2.4.2. Patients with large bowel disorders

### 2.4.2.1. Detection of TRPV1

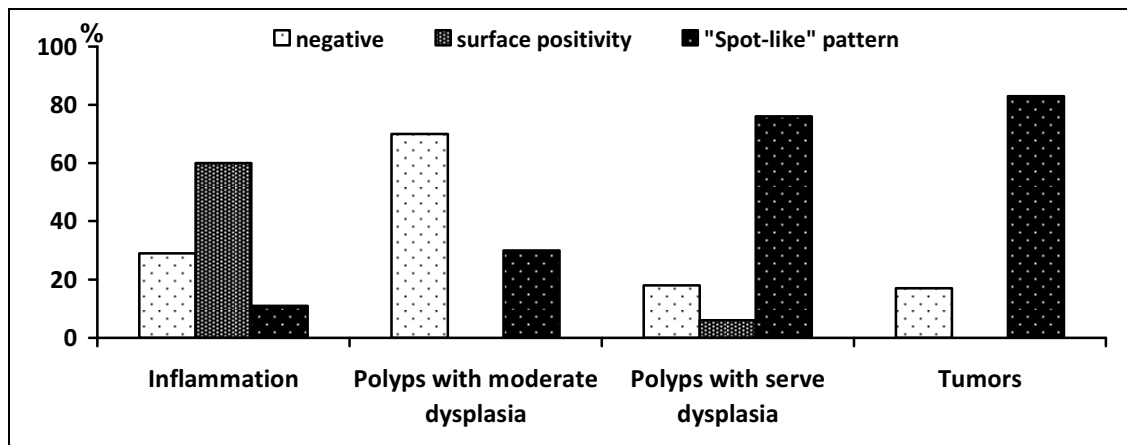
The presence of TRPV1 was detected in colon mucosa of patient with inflammation and polyps (Fig. 2.11), inflammation and dysplasia (Fig. 2.12). Figure 2.13 shows the summary of the immunohistochemical distribution of TRPV1 in the colon diseases (inflammation, polyps, dysplasia, tumor), which indicates the positivity, negativity and spot-like pattern.



**Figure 2.11.** Immundistribution of TRPV1 in the colon mucosa of a patient with moderate dysplastic polyp (A) and a patient with inflammatory bowel disease (B). Arrows show the immunsigns in the mucosa.



**Figure 2.12.** Immunmorphology of TRPV1 in the colon mucosa of a patient with inflammatory bowel disease (A) and a patient with severe dysplastic polyp (B). The picture B demonstrates the “spot like” immunsigns at the paranuclear area. Arrows show the immunsigns in the mucosa.

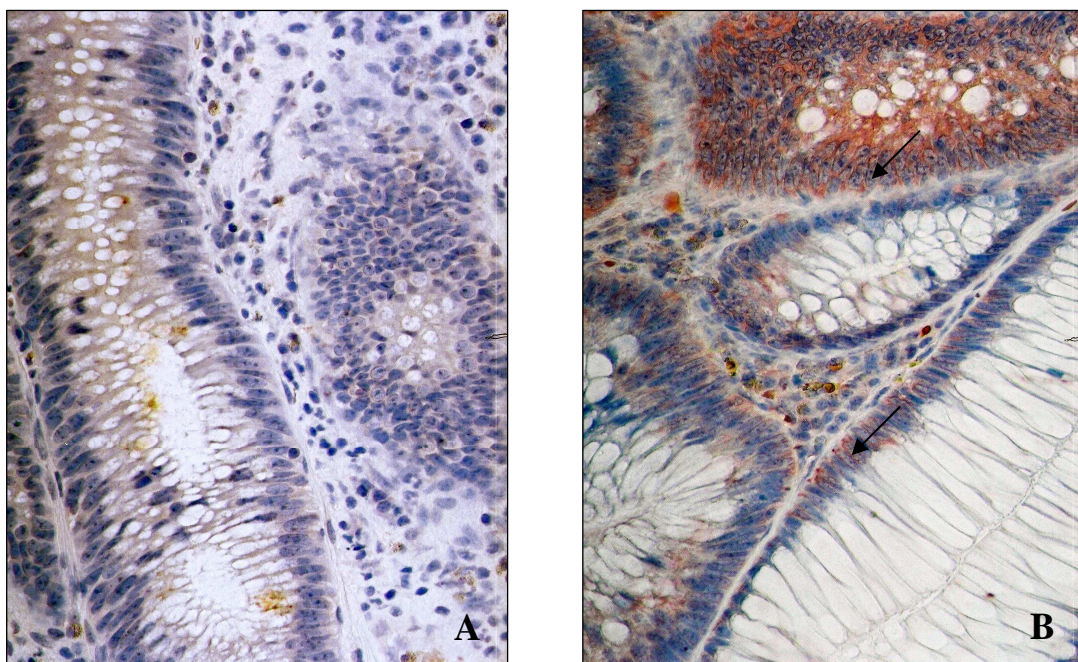


	Negative	Positive	„Spot-like” pattern
Inflammatory bowel diseases	29.4% (5)	59.8% (10)	11.8% (2)
Polyps with moderate dysplasia	70.6% (12)	0% (0)	29.4% (5)
Polyps with severe dysplasia	17.6% (3)	5.9% (1)	76.5% (13)
Colon adenocarcinoma	16.7% (1)	0% (0)	83.3% (5)

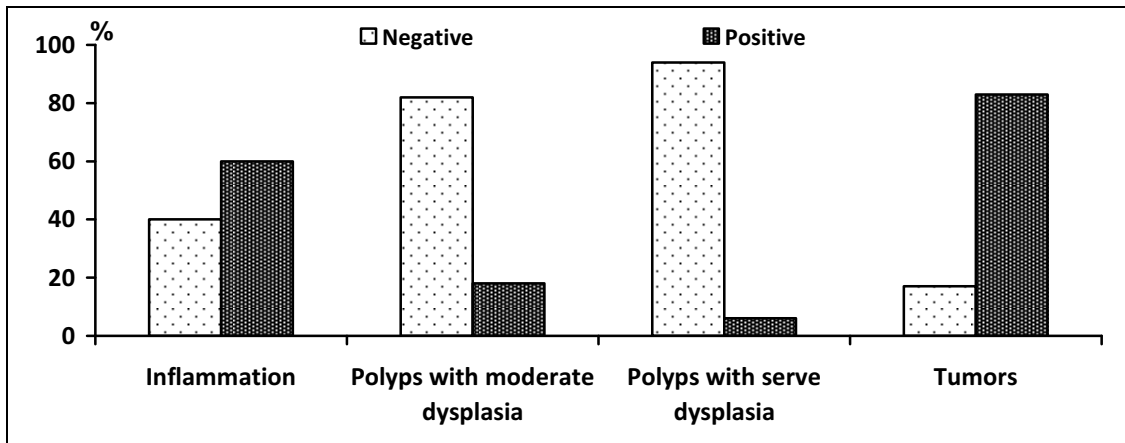
**Figure 2.13.** Summary of TRPV1 distribution in the colon mucosa of patients with different large bowel diseases. The numbers between the parentheses represent the number of the patients.

#### 2.4.2.2. Detection of CGRP

The immunohistochemical negativity and positivity are demonstrated in the colon mucosa of patients with polyps and inflammatory bowel disease (Fig. 2.14). The summary of the CGRP positivity and negativity is given in Fig. 2.15.



**Figure 2.14.** Demonstration of negativity (A) and positivity (B) of CGRP by immunomorphology in colon mucosa of patients with colon diseases. Arrows show the immunosigns in the mucosa.

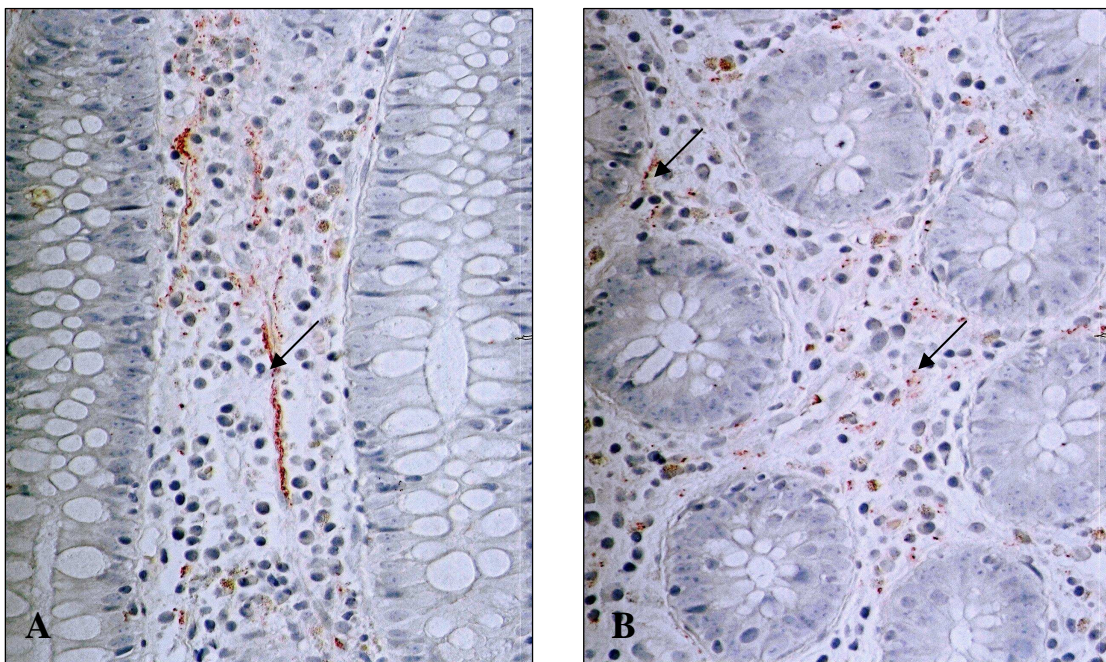


	Negative	Positive
Inflammatory bowel diseases	40.2 % (7)	59.8 % (10)
Polyps with moderate dysplasia	82.4 % (14)	12.6 % (3)
Polyps with severe dysplasia	94.1 % (16)	5.9 % (1)
Colon adenocarcinoma	16.7 % (1)	83.3 % (5)

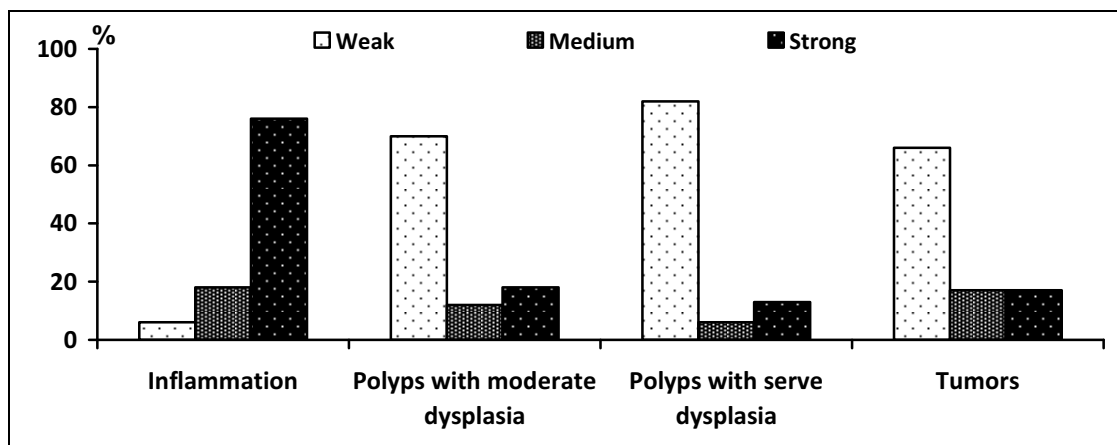
**Figure 2.15.** Summary of CGRP distribution in the colon mucosa of patients with different large bowel diseases. The numbers between the parentheses represent the number of the patients.

#### 2.4.2.3. Detection of SP.

Immunohistochemical positivity is demonstrated in the colon mucosa (Fig. 2.16). Figure 2.17. summarizes the results of immunohistochemical distribution in the colon inflammation, polyps, dysplasia and tumors.



**Figure 2.16.** Immundistribution of SP can be observed in a longitudinal (A) and in a cross section (B) of the colon mucosa in a patient with inflammatory bowel disease. Arrows show the immunsigns in the mucosa.



	Weak	Medium	Strong
<b>Inflammatory bowel diseases</b>	5.9% (1)	17.6% (3)	76.5% (13)
<b>Polyps with moderate dysplasia</b>	70.6% (12)	11.8% (2)	17.6% (3)
<b>Polyps with severe dysplasia</b>	82.4% (14)	5.9% (1)	11.8% (2)
<b>Colon adenocarcinoma</b>	66.6% (4)	16.7% (1)	16.7% (1)

**Figure 2.17.** Summary of SP distribution in the colon mucosa of patients with different large bowel diseases. The numbers between the parentheses represent the number of the patients.

Table 2.2 summarizes the immunohistochemical distribution of TRPV1, CGRP and SP in colon inflammation, polyps with moderate and severe dysplasia and tumors.

**Table 2.2.** Summary of the immunohistochemical changes in the colon mucosa of patients with different large bowel disorders.

	TRPV1	CGRP	SP
<b>Inflammatory bowel disease</b>	+	+	+
<b>Polyps with moderate dysplasia</b>	-	-	-
<b>Polyps with severe dysplasia</b>	+	-	-
<b>Colon adenocarcinoma</b>	+	+	-

All three examined immunohistochemical changes are present in inflammation. TRPV1 is also present in dysplasia and tumors, and CGRP in tumors; however, SP is not typically present in polyps with moderate and severe dysplasia and tumors of the colon.

## 2.5. Discussion and conclusion

Both the afferent and efferent vagal nerves are involved in the development of different gastrointestinal disorders (inflammation, dysplasia, polyps, cancers). The line intact tissue → inflammation → hyperplastic polyps → dysplastic polyps → tumor represents the biochemical, morphological and clinical trends for the development of malignant diseases. Of course the etiological factors are only suspected, but not exactly known in inflammation, hyperplasia, dysplasia, polyps and cancer. On the other hand, the stomach (from the proximal part) and colon (from the distal part) of the gastrointestinal tract were studied in this presentation.

The participation of activated protein C (Leiden mutation), decrease of serum vitamin A and zeaxanthin have been demonstrated in chronic inflammatory bowel diseases, oesophageal, stomach, liver, pancreatic and colorectal cancers (40). No change was found both in the activated protein C and retinoids in acute gastritis and hepatitis (40). Together, these results proved the participation of some hereditary (activated protein C) and nutritional environmental (retinoids) factors in the development of chronic inflammatory diseases and oesophageal, gastric, liver, pancreatic and colon cancers (41).

In our present study we evaluated the capsaicin-sensitive afferent nerves (TRPV1 and liberated neuropeptides CGRP, SP). We did not have any earlier information on the possible role of capsaicin-sensitive afferent nerves in the development of inflammation, hyperplasia, dysplasia, polyps and tumors of stomach and colon in patients, as we could not find relevant literature citation(s) (42). Our other observations clearly proved that during development of omeprazole-induced gastric protection an overlap exists between the capsaicin-sensitive afferent nerves and efferent nerves of the vagal nerve (39).

Our results prove the participation of capsaicin-sensitive afferent nerves in the development of inflammation, hyperplasia, dysplasia, polyps and tumors both in the proximal (stomach) and distal (colon) parts of the GI tract in humans.

Up to now, all of the researchers emphasized the role of capsaicin-sensitive afferent nerves in the development of different inflammatory processes. Our present results clearly prove that the capsaicin sensitive-afferent nerves (and neuropeptides released by them) participate in the development of chronic inflammation, hyperplasia, dysplasia, polyps and cancers in the stomach and colon in patients. To date the details of these

mechanisms are unknown. However, taken together these results prove the role of capsaicin-sensitive afferent nerve in the development of these diseases. These afferent nerves can be modified by different chemicals, originating from the human body and from different foods.

In consequence of these studies we can conclude that:

1. Capsaicin-sensitive afferent nerves and neuropeptides released by them take part in the development of different human gastrointestinal disorders.
2. The immunohistochemical distribution of TRPV1, CGRP and SP differs in the upper (gastric) and distal (colon) parts of the gastrointestinal tract (in inflammation, hyperplasia, dysplasia, ulcer, polyps, cancers).

These observations show only a momentary role of the capsaicin-sensitive nerves in the development of these GI disorders but the involvement/importance of an etiological factor or the effect of a treatment could not be observed/found out.

Other observations have to be made

1. to demonstrate immunodistribution of capsaicin-sensitive nerves (as TRPV1) and liberated neurotransmitters (SP, CGRP) in GI disorders (like gastritis), which have the same pathomorphological appearance but could be caused by different factors – causative factor dependence?;
2. to approach the possible gastric mucosal defensive mechanisms of capsaicin-sensitive afferent nerves (immunohistochemical distribution of TRPV1, CGRP, SP) in the development of gastritis and its treatment.

### **3. "Participation of capsaicin-sensitive afferent nerves in the gastric mucosa of patients with *Helicobacter pylori* positive or negative chronic gastritis"**

#### **3.1. Introduction**

Gastritis is a pathomorphological appearance of inflammation in the gastric mucosa. Acute and chronic gastritis can be differentiated by the reasons of the development and the process of the disease. The chronic gastritis may be caused by different factors like as *H. pylori* infection, bacterial overgrowth in a hypochlorhydric stomach, autoimmun mechanisms or chemical agents like long-term nonsteroid anti-inflammatory drug (NSAID) treatment or bile reflux (43, 44). Nowadays, the importance of *H. pylori* infection is rising as the main causative factor in gastric diseases in humans. This bacterium is highly prevalent in many countries (45) and it increases the risk for development of gastric and duodenal ulcer disease, gastric cancer, and gastric mucosa-associated lymphoid tissue (MALT) lymphoma (46, 47, 48, 49, 50, 51).

The dysfunction of the gastrointestinal mucosal defense mechanisms is also involved in the development of gastric diseases. The capsaicin-sensitive afferent nerves take part in gastric mucosal protection in animals (9, 19, 52), in human healthy subjects (26, 33, 53) and the presence of these fibers is proved in the development of human gastrointestinal disorders including gastritis, peptic ulcer, polyp without and with dysplasia, tumor and inflammatory bowel diseases (42, 54). The mechanisms which these neurons express their effects are complex but widely investigated. The capsaicin-sensitive afferent nerves contain a temperature-gated ion channel called capsaicin receptor or TRPV1, which receptor is sensitive for noxious heat, protons, vanilloids (capsaicin) and endogenous ligands (anadarmide, N-oleodopamine and lipoxygenase products) (12). By stimulating these afferent fibers with capsaicin, four response stages (excitation, sensory-blocking, long-term selective neurotoxic impairment and irreversible cell destruction) are developing depending on the dose and duration of exposure of the drug (11). Small dose of capsaicin ( $\mu\text{g}/\text{kg}$ ) causes excitation on the nerve endings and neuropeptides (SP, CGRP and SS) are released (8, 13, 14). These mediators could increase the mucosal blood flow by vasodilatation (55), activate mast cells and immuncells in the mucosa (56), they are involved in drug effect (34, 39) and



somatostatin could elicit systemic anti-inflammatory and analgetic „sensory functions”. The immunodistribution of neuropeptides (SP, VIP, NPY, SS, GAL) released from the sensory neurons and its neuro-immun function are known in *H. pylori* positive gastritis but not examined in gastritis without this infection (57).

### 3.2. Aim

The aims of the study were:

1. To collect patients suffered from gastritis,
2. To divide them into groups according to the presence or absence of *H. pylori* infection,
3. To examine the role of the capsaicin-sensitive afferent nerves in the development of chronic gastritis by detecting the immunodistribution of the capsaicin receptor and the liberated neurotransmitters (CGRP and SP),
4. To obtain the difference in the immunostainings between the *H. pylori* positive or negative chronic gastritis.

### 3.3. Materials and methods

The symptoms of the patients suffered from chronic gastritis with or without *Helicobacter pylori* infection (21 *H. pylori* positive, 30 *H. pylori* negative) were unspecific (gastric discomfort sensation, nausea, loss of appetite, vomiting). They went over physical, laboratory, ultrasonography, endoscopic and histological examinations at the First Department of Medicine, Medical and Health Centre, University of Pécs.

Twenty peoples with functional dyspepsia (all of them went over the above mentioned medical, laboratory, iconographic and histological examinations and all of these examinations indicated absolutely negative results) were taken as healthy controls. The age of patients was 39 to 68 years 22 males, 29 females, and 10 males and 10 females in the group of functional dyspepsia.

The gastric biopsies were collected from the hyperaemic areas of the corpus and antrum of the stomach by eosophago-gastro-bulboscopy. The *Helicobacter pylori* infection was detected by using [<sup>13</sup>C] urea breath test ([<sup>13</sup>C] UBT), rapid urease test and specific histological examinations. The gastric tissue samples were analyzed in the Department of Pathology, and classified into different groups of chronic gastritis according to the Sydney's System (58). The biopsies showed moderate and severe activity of

inflammation. The groups of patients with chronic gastritis and histologically healthy persons were established on the opinion of an independent histopathologist.

The immunohistological studies were carried out on formalin fixed, paraffin embedded tissue samples using the peroxidase-labeled polymer method (Lab Vision Corp., Fremont, USA). The SP was detected by the NC1/34 HL rat monoclonal antibody, the TRPV1 receptor and CGRP were labeled using polyclonal rabbit antisera (all from Abcam Ltd., UK Cambridge) (Table 3.1.).

**Table 3.1.** Used primary antisera

Antisera	Abbreviation	Species	Dilution	Source
Calcitonin gene-related peptide	CGRP	Rabbit	1:200	Abcam, Cambridge, UK
Substance P	SP	Rat	1.200	Abcam, Cambridge, UK
Transient receptor potential vanilloid 1	TRPV1	Rabbit	1:400	Abcam, Cambridge, UK

The immunohistochemical analysis was assessed by light microscopy (Olympus). The TRPV1 and CGRP were detected as positive or negative, meanwhile the SP immunodistribution was characterized by using a „SP-index”. This index was calculated by counting immunopositive spots in at least five high magnification fields. In fields without immunostaining, the score was zero, fields’ containing only one positive spots, the score was one and fields with two or more stained elements were scored two. The total score in one specimen was divided by the number of the scanned field to obtain the SP-index. Based on these results, biopsies were classed into three categories; weak, medium and strong (Table 3.2.).

**Table 3.2.** Semiquantitative quantitation of immunohistochemical staining of SP in the gastric mucosa of healthy subjects and of patients with chronic gastritis.

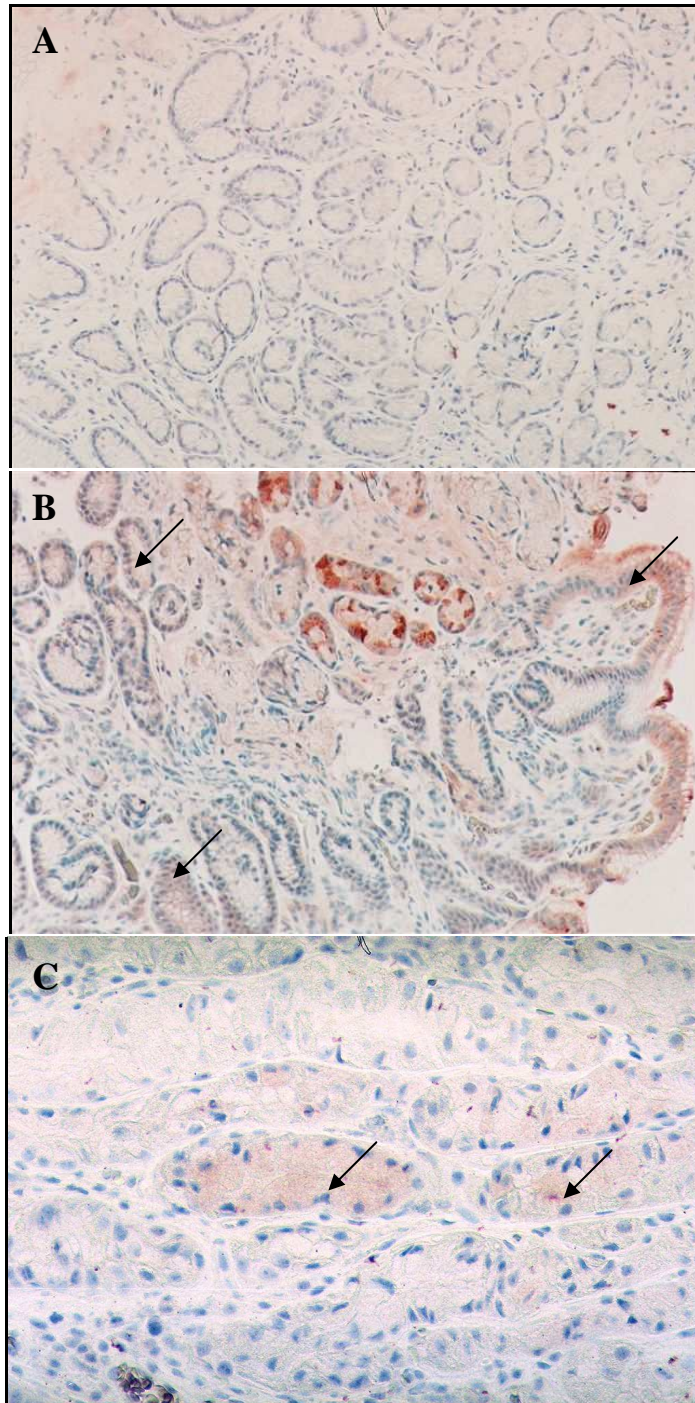
SP evaluation	SP-index
Weak	$<0,5$
Medium	$0,5 \leq \text{and} < 1$
Strong	$1 \leq$

The human examinations were permitted by the Regional Ethical Committee of University Pécs, Hungary. Written informed consent was obtained from all participants.

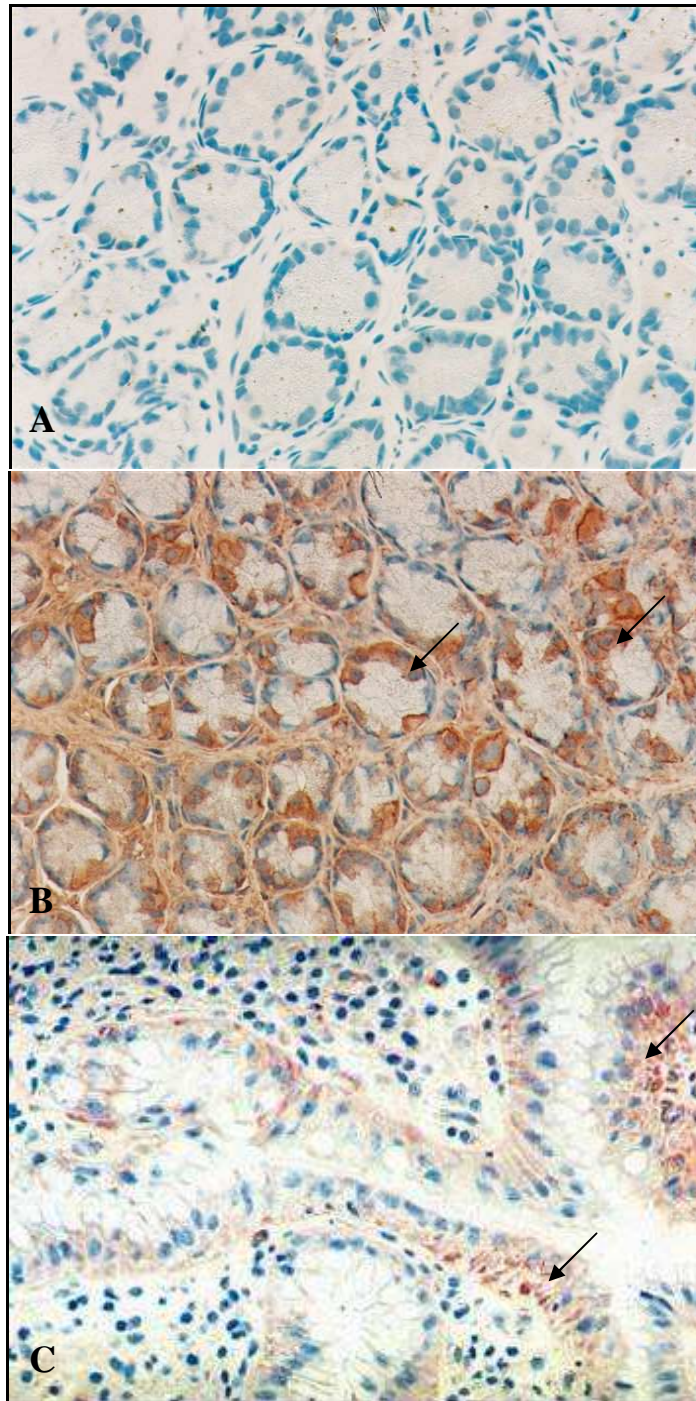
*Statistical analysis:* TRPV1 and CGRP were statistically evaluated by  $\chi^2$ -probe, meanwhile the SP results were semiquantitatively evaluated by Mann-Whitney’s U test. The results were taken to be significant, if P values were  $\leq 0,05$ .

### 3.4. Results

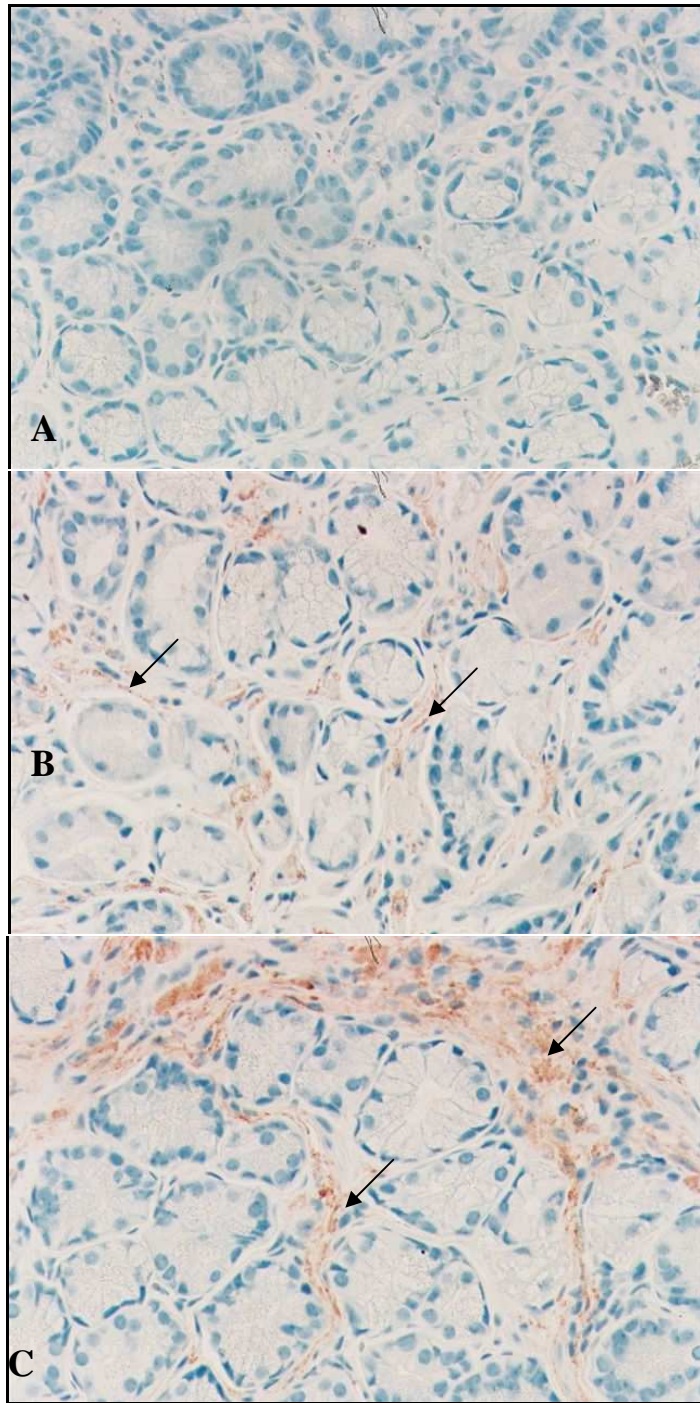
The results are presented by the typical pictures of the immunomorphological appearance of the studied receptor and mediators in the gastric mucosa in human healthy subjects and in patients with chronic gastritis (Figs. 3.1-3).



**Figure 3.1.** Immundistribution of TRPV1 in the gastric mucosa of a healthy subject (A) and of patient with *H. pylori* negative (B) and *H. pylori* positive (C) chronic gastritis. Arrows show the immunsigns in the mucosa.



**Figure 3.2.** Immundistribution of CGRP in gastric mucosa of a healthy subject (A), of patient with *H. pylori* negative (B) and *H. pylori* positive (C) chronic gastritis. Arrows show the immunsigns in the mucosa.

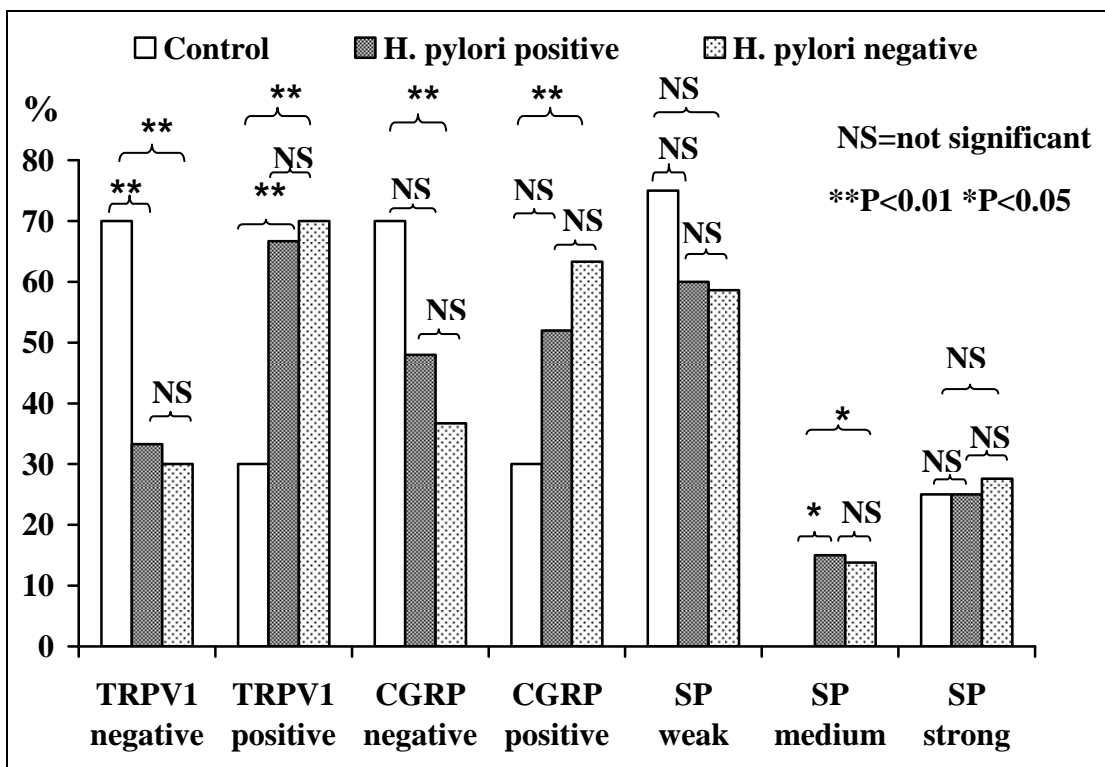


**Figure 3.3.** Immundistribution of SP in gastric mucosa of a healthy subject (A) and of patient with *H. pylori* negative (B) *H. pylori* positive (C) chronic gastritis. Arrows show the immunsigns in the mucosa.

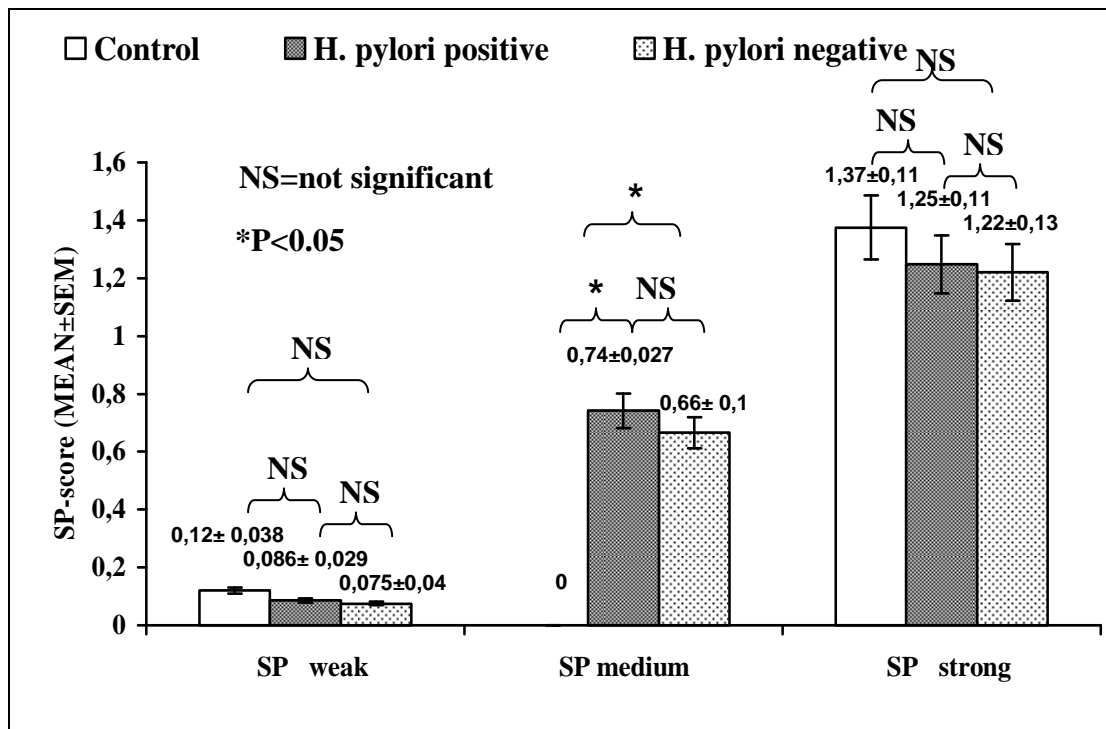
The summary of the immunohistochemical results is demonstrated by Table 3.3 and Figs. 3.4-5 show the SP-scores (MEAN±SEM) in patients with chronic gastritis and in healthy persons.

**Table 3.3.** Summary of results of immunohistochemical examinations of TRPV1, CGRP and SP in the gastric mucosa of human healthy subjects and of patients with chronic gastritis. The numbers between the parentheses represent the number of the patients.

	TRPV 1 positive	TRPV 1 negative	CGRP positive	CGRP negative	SP weak	SP medium	SP strong
<b>Control</b>	20 % (4)	80 % (16)	30 % (6)	70 % (14)	75 % (15)	0 % (0)	25 % (5)
<b><i>H. pylori</i> positive</b>	66.7 % (14)	33.3 % (7)	52 % (11)	48 % (10)	60 % (12)	15 % (3)	25 % (5)
<b><i>H. pylori</i> negative</b>	70 % (21)	30 % (9)	63.3 % (19)	36.7 % (11)	58.6 % (17)	13,8 % (4)	27.6 % (8)



**Figure 3.4.** The results of immundistribution of TRPV1, CGRP and SP in the gastric mucosa of patients suffered from *H. pylori* positive or negative chronic gastritis and in healthy subjects.



**Figure 3.5.** SP-score values in the gastric mucosa of patients with *H. pylori* positive or negative chronic gastritis and of healthy subjects.

In the TRPV1 positive cases of chronic gastritis with or without *H. pylori* infection and in healthy subjects, the immunostaining was detected as fine granular cytoplasmic immunosigns in the epithelial cells at gastric mucosa (Fig. 3.1).

The TRPV1 positive cases of chronic gastritis were significantly higher ( $P < 0.01$ ) extent than in controls meanwhile no significant difference was detected in the immunomorphology of TRPV1 between the patients with *H. pylori* negative or positive chronic gastritis.

The characteristic immunmdistribution of CGRP was fine granular cytoplasmic positivity in the epithelial cells at gastric mucosa of patients with chronic gastritis with or without *H. pylori* infection and in healthy persons (Fig. 3.2).

In the immunhistological distribution of CGRP, significant changes could be observed between the healthy and *H. pylori* negative chronic gastritis ( $P < 0.01$ ) and no significant difference was found in the two types (*H. pylori* negative or positive) of chronic gastritis. Although the count of the positive tissue samples increased in *H. pylori* positive gastritis, it did not reach significantly different level compared to healthy controls.

The immunomorphology of SP was detected as granular small spot-like signals along the mucosal blood vessels at gastric mucosa of healthy subjects and of patients with chronic gastritis with or without presence of *H. pylori* infection (Fig. 3.3).

No significantly change could be observed in the amount of weak and strong SP-score between the healthy subjects and the patients with chronic gastritis meanwhile the medium cases of SP immunohistological samples appeared in chronic *H. pylori* negative or positive gastritis.

### **3.5. Discussion and conclusion**

The inflammation of the gastric mucosa is a general first (but not specific) reaction to different physical (irritation), chemical (ethanol, nonsteroid anti-inflammatory drugs, reserpine), bacterial (*H. pylori* and other strains) and viral stress. The capsaicin-sensitive afferent nerves take place in the integrity of gastric mucosa in animals and in human beings (19, 26, 33) by liberating mediators (SP and CGRP) from the axon terminals (“dual hypothesis”) (8).

The roles of the capsaicin-sensitive afferent nerves and these mediators were known in patients with different gastrointestinal disorders like chronic gastritis, peptic ulcer, erosion and tumors (42, 54, 57).

It was suggested by us that the participation of capsaicin-sensitive afferent nerves depends on the causative factor to produce gastritis in patients and of course the *H. pylori* infection was especially suggested as a causative factor to produce gastritis in patients. To detect the probable “causative factor dependent” functions of capsaicin-sensitive nerves, the immunohistochemical distributions of capsaicin receptor, CGRP and SP were studied in patients with chronic *H. pylori* positive and negative gastritis and in histologically healthy subjects.

Based on our observations, the results can be summarized in two parts:

- 1.) The capsaicin-sensitive nerves take place in the development of chronic gastritis which is demonstrated by the significantly increased immunohistochemical expression of capsaicin receptor, CGRP and SP in the gastric mucosa of patients with chronic gastritis (with or without *H. pylori* infection) (compared to the healthy subjects).
- 2.) The involvement of capsaicin-sensitive afferent nerves in human chronic gastritis does not depend on the presence of *H. pylori* infection, indicated by the observed



same immunohistochemical appearance of TRPV1, CGRP and SP in the gastric mucosa and the similar ratio between the positive and negative cases of the studied receptor and mediators in patients suffered from *H. pylori* positive or negative chronic gastritis.

In conclusion, the increased expression of capsaicin receptor, CGRP and SP could be one of compensatory mechanisms produced by the capsaicin-sensitive afferent nerves in patients with chronic gastritis and this first tissue reaction to “noxious agents” do not depend on the presence of *H. pylori* infection.

The involvement of the capsaicin-sensitive afferentation is not known in the therapeutic effects of drug in humans so other observation have to be proposed.

## **4. "Capsaicin-sensitive afferentation represents an indifferent defensive pathway from eradication in patients with *Helicobacter pylori* gastritis"**

### **4.1. Introduction**

The gastric lesions are caused by the disruption of a balance between the aggressive and defensive factors. The most common "aggressive" pathogen bacterium of/in the stomach is the *H. pylori* (59). The importance of the *H. pylori* infection inhere not only its widely spreading but also it is etiologic factor of different human gastrointestinal disorders (acute gastritis, chronic gastritis, gastric ulcer, gastric MALT lymphoma, gastric adenocarcinoma, duodenal ulcer) (45, 46, 47, 48, 49, 50, 51, 60, 61). The eradication of this organism has been associated with histological improvement of gastritis (8).

On the other side, capsaicin-sensitive afferentation is one of the defensive mechanisms. The role of these nerves has been demonstrated in gastric mucosal protection by preventing of the drugs-induced mucosal injury in animals (9, 19, 52) and by decreasing of the amount of IND-induced gastric microbleedings in humans healthy subjects (26, 33, 56).

A temperature-gated nonselective cation channel (called capsaicin receptor or TRPV1) is the "capsaicin-sensitive" part of these nerves. This receptor is sensitive not only for capsaicin and other vanilloids but also for protons, noxious heat and endogenous ligands (anadarmide, N-oleodopamine) and lipoxygenase products (12). The TRPV1 was detected in the area postrema and in the nucleus tractus solitarii where the afferent fibers of the vagal nerve terminate. The nervus vagus consists of 10 % efferent nerves, 90 % of afferent nerves and 10 % of the afferent nerves represents the capsaicin-sensitive afferentation of the vagal nerve. The amount of the efferent nerves and the capsaicin-sensitive afferent nerves are roughly the same amount in the vagal nerve.

Capsaicin exposure exerts various responses in these afferent nerves depending on the dose and the exposure duration (excitation, sensory-blocking, long-term selective neurotoxic impairment and irreversible cell destruction) (11). During administration of small doses of capsaicin (from ng/kg to µg/kg body weight) neurotransmitters (SP, SS, CGRP etc.) are released from this nerve endings (8, 13, 14). These mediators are

responsible for the increase of mucosal blood flow by vasodilatation (55), activation of mast cells and immuncells in the mucosa (56, 57).

The presence of this receptor and of released neurotransmitters has been studied in the development of human gastrointestinal disorders including gastritis, peptic ulcer, polyp, tumor and inflammatory bowel diseases by immunohistology (34, 42, 54). In our recent work significant changes were observed in the presence of TRVP1, CGRP and SP in patients with chronic *H. pylori* positive gastritis and in histologically healthy subjects but no change could be detected between the patients who suffered from chronic gastritis without or with *H. pylori* infection (62).

The effects of omeprazole and omeprazole-like compounds have also been evaluated on changes of TRVP1, SP and CGRP immunodistribution in rats (39).

#### **4.2. Aim**

The aim of our present study was to analyze the role of capsaicin afferent nerves (e.g. immunohistochemical distribution of TRVP1, CGRP, SP) in the gastric mucosa of the patients with chronic gastritis produced by *H. pylori* before and after eradication treatment.

#### **4.3. Materials and methods**

The observations were carried out in 38 persons, including 20 healthy subjects and 18 patients with *H. pylori* positive gastritis.

Eighteen patients with *H. pylori* positive chronic gastritis underwent physical, laboratory, ultrasonography, endoscopic and histological examination at the Department of Medicine and Gastroenterology, Markusovszky Teaching Hospital, Szombathely (Hungary). The age of patients (6 males, 12 females) was 39 to 68 years (mean = 56.4 years) and a questionnaire was used to determine the patients' symptoms.

Twenty peoples with functional dyspepsia (all of them went over the above mentioned medical, laboratory, iconographic and histological examinations and all of these examinations indicated absolutely negative results) were taken as healthy controls. The age of patients (10 males and 10 females) was 41 to 67 years (mean = 52.1 years) in the group of functional dyspepsia.

The *H. pylori* infection was detected before and after the eradication therapy using the [<sup>13</sup>C] UBT, rapid urease test, Warthin-Starry silver staining and specific histological examinations.

The gastric biopsies from patients with *H. pylori* positive chronic gastritis were collected from the hyperemic areas of the gastric corpus and antrum by gastroscopy before and after eradication therapy. The gastric tissue samples were analyzed in the Department of Pathology of Markusovszky Teaching Hospital, Szombathely (Hungary) and classified into different groups of chronic gastritis according to the Sydney's System (30). The groups of patients with chronic gastritis and histological healthy persons were established by an independent experienced GI pathologist.

*H. pylori* positive patients underwent 7 days long eradication treatment with the combination of double dose PPI (pantoprazole 2 × 40 mg/d), amoxicillin (1000 mg twice daily) and clarithromycin (500 mg twice daily) according to current European guidelines (63). After this 1 week combination therapy, patients continued to take a normal dose of PPI for another week. The time period between the first and control gastroscopy was 6 weeks. Similar gastric biopsies were taken from the corpus and antrum of healthy subjects.

The immunohistological studies were carried out on formalin fixed, paraffin embedded tissue samples of gastric mucosa using the peroxidase-labeled polymer method (Lab Vision Co., Fremont, USA). SP was detected by the NC1/34 HL rat monoclonal antibody, the TRVP1 and CGRP were labeled using polyclonal rabbit antisera (all from Abcam Ltd., UK Cambridge).

The immunohistochemical examinations were performed in the Histopathology Ltd., Pécs (Hungary).

The human examinations were permitted by the Regional Ethical Committee of University Pécs, Hungary. Written informed consent was obtained from all participants. TRVP1, SP and CGRP were statistically evaluated by  $\chi^2$ -probe. The results were taken to be significant, if *P* values were  $\leq 0.05$ .

#### 4.4. Results

Before *H. pylori* eradication, the symptoms of patients with *H. pylori* positive chronic gastritis were unspecific: epigastric pain (14/18, 77%), heartburn (13/18, 72%), nausea/vomiting (9/18, 50%) abdominal expansion (9/18, 50%), constipation (6/18, 38%).

Moderate and severe activity of inflammation could be observed in the gastric biopsies of patients with *H. pylori* positive chronic gastritis before eradication treatment.

The *H. pylori* eradication therapy was successful in 16 of 18 patients (89%).

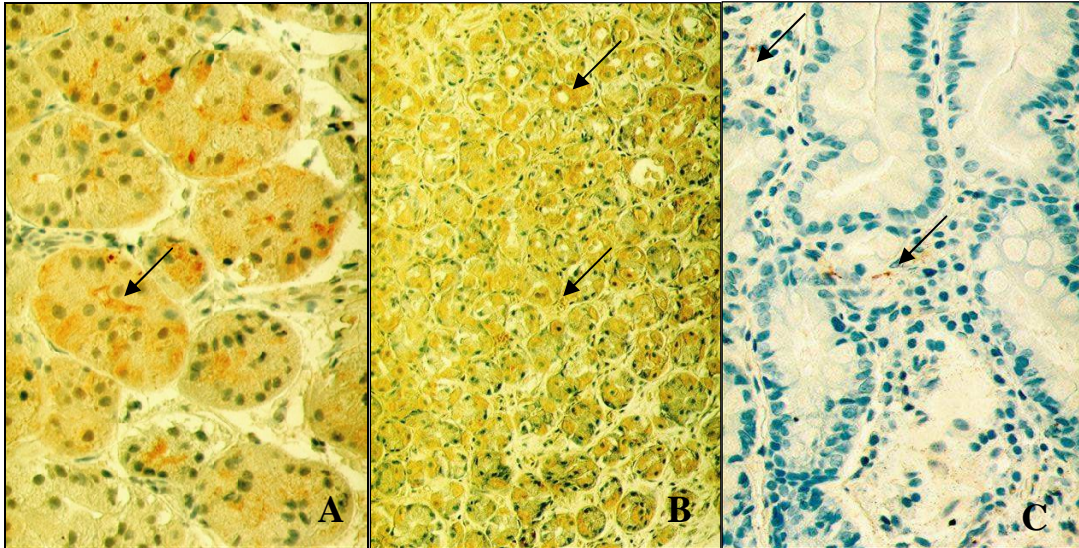
After eradication, symptoms were found to remain moderate in seven patients (7/18, 39%) and 11 patients (11/18, 61%) had no complaints after treatment.

Histologically healthy gastric mucosa could be detected only in 4 cases of the control biopsies and in 14 cases the appearance of chronic gastritis was moderated.

Fine cytoplasmic TRVP1 positivity was detected in 37% (7/20) of healthy subjects, whereas *H. pylori* positive gastritis patients were 89% (16/18,  $P < 0.001$ ) positive before and 72% (13/18  $P < 0.03$ ) positive after eradication therapy.

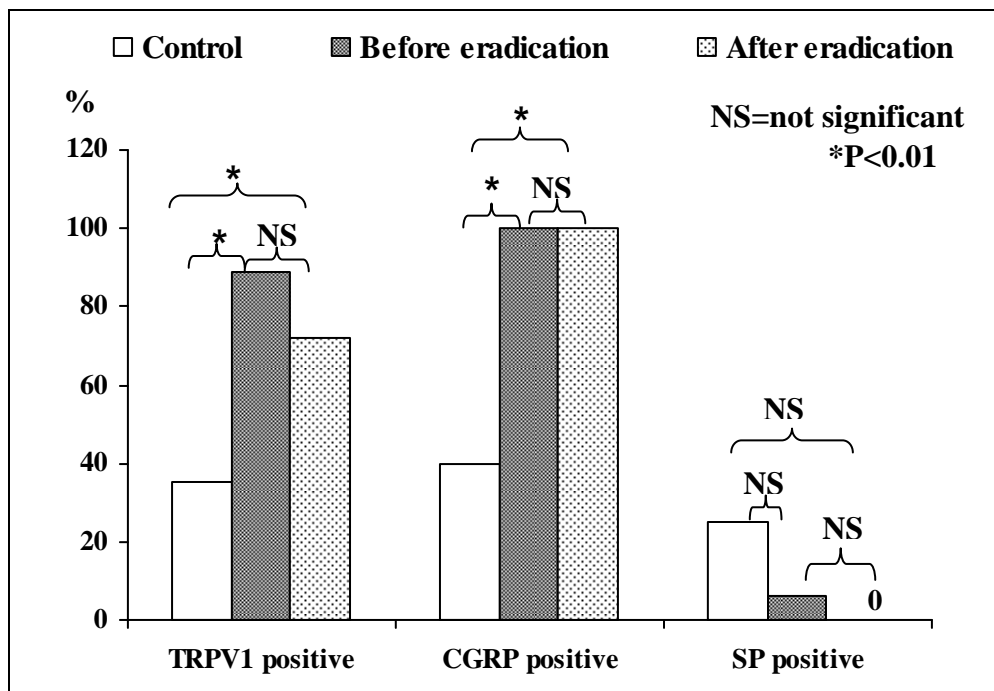
CGRP staining showed fine positively stained cytoplasmic granules distributed in both the epithelial cells of gastric mucosa of patients with *H. pylori* positive chronic gastritis and in histologically healthy persons (Figure 4.1.B). Immunohistochemistry for CGRP was positive in 100% (18/18,  $P < 0.001$ ) of patients both before and after eradication (18/18,  $P < 0.001$ ). In the mucosa of healthy individuals this staining was found to be positive in 40% (8/18) of controls.

SP was detected as small granular spot-like signals localized along the mucosal blood vessels (Figure 4.1.C). The SP immune-staining was positive in 25% (3/20) of control persons, and in 5.5% (1/18,  $P > 0.05$ ) before and 0% (0/18,  $P > 0.05$ ) after eradication in the gastric mucosa.



**Figure 4.1** Immune-staining for TRPV1 (A), CGRP (B) and SP (C) in the gastric mucosa of patients with *H. pylori* positive chronic gastritis ( $\times 100$ ). The arrows show the immunosigns in the mucosa.

The results of the immunohistological examinations for TRVP1 and mediators (CGRP and SP) are summarized in Table 4.1. Interestingly, when compared to normal mucosa, the presence of SP decreased in the mucosa obtained from patients with *H. pylori* positive chronic gastritis before and after eradication treatment (Figure 4.2).



**Figure 4.2.** Changes in the immunohistochemical distribution of capsaicin receptor (TRPV1), CGRP and SP in patients with *H. pylori* positive chronic gastritis before and after eradication.

**Table 4.1** Summary of the presence of capsaicin receptor, CGRP and SP in the gastric mucosa of patients with chronic *H. pylori* positive gastritis, before and after eradication therapy, obtained by immunohistochemistry. The numbers between the parentheses represent the number of the patients.

	TRPV1		CGRP		Substance P	
	Positive	Negative	Positive	Negative	Positive	Negative
<b>Before eradication</b> (n=18)	88.89 % (16)	11.11 % (2)	100 % (18)	0 % (0)	5.56 % (1)	94.44 % (17)
<b>After eradication</b> (n=18)	72.22% (13)	17.78% (5)	100 % (18)	0 % (0)	0 % (0)	100 % (18)
<b>Control group</b> (n=20)	35 % (7)	65 % (13)	40 % (8)	60 % (12)	25 % (5)	75 % (15)

#### 4.5. Discussion and conclusion

The possible role(s) of the capsaicin-sensitive afferent vagal nerve has been studied by our team since 1980 under physiological and different pathological conditions in animal experiments (9, 19, 34), healthy subjects (64) and in patients with different gastrointestinal disorders (54, 62).

The presence of TRPV1, CGRP significantly increased in *H. pylori* positive chronic gastritis compared with gastric mucosa of healthy subjects. The data suggested that the TRPV1, CGRP could be involved in the development of human chronic gastritis; however no significant changes were obtained after classical *H. pylori* eradication treatment. SP levels decreased in patients with *H. pylori* chronic gastritis and its value was not changed by eradication treatment.

Histologically healthy gastric mucosa could be detected in only 22% of patients at 6 weeks after classical eradication treatment. It was interesting to note, however, that the distribution of gastric mucosal TRPV1, CGRP and SP did not change in *H. pylori* positive gastritis after classical eradication treatment.

How can we explain this unchanged distribution?

We might need to start with the observed facts:

1. TRPV1, CGRP and SP can be immunohistologically detected in rat and human gastric mucosa under healthy and different pathological circumstances.

2. The changes in expression of TRPV1, CGRP and SP are a consequence of activation in capsaicin-sensitive afferent nerves.
3. The presence of *H. pylori* was proved in all patients with chronic gastritis in our study.
4. The eradication of *H. pylori* was successfully carried out, and that was associated with a significant decrease in patients' complaints.
5. The gastric mucosa normalized in 22% of patients; and 78% of patients demonstrated only moderate histological signs of gastritis in their gastric mucosa after eradication treatment.
6. Independently from the proof that chronic gastritis is associated with *H. pylori* infection, the histological picture of gastric mucosa indicates only a moderate remission.

We are able to explain the unchanged immunohistochemical distribution of TRPV1, CGRP and SP of *H. pylori* positive chronic gastritis after successfully eradication treatment as follows:

- The time period (6 weeks) after eradication is enough time for decreasing the patients' complaints but not enough for the complete histological recovery and healing of chronic *H. pylori* (in terms of histology and immunohistology) in patients.
- It can be suggested that other permanent factors (stress, drugs), not responding to the *H. pylori* eradication therapy, also play a part in the development of chronic gastritis; this is in accordance with our further observations.
- The immunohistological distribution of TRPV1, CGRP and SP seems to be independent of the chronic gastritis produced by different physical, chemical, bacteriological, immunological agents.
- A low percentage of the participants was refractory to the eradication therapy so that the persistent *H. pylori* infection before and after eradication, could maintain the same immunohistochemical appearance and the same inflammatory response.

Earlier, we demonstrated that the amount of vanilloid receptor, CGRP and SP increased in patients with chronic gastritis; however, no differences were obtained in their presence in gastric mucosa of *H. pylori* positive and *H. pylori* negative patients (62). These results clearly indicate that the immunohistochemical distribution of vanilloid



receptor, CGRP and SP during the classical eradication treatment in patients with *H. pylori* positive chronic gastritis is independent from the eradication treatment. These facts suggest that the function of capsaicin-sensitive afferent nerves is also independent. Capsaicin is able to reduce the IND-induced gastric microbleeding in human healthy subjects. The involvement of TRVP1, CGRP and SP in different gastrointestinal disorders shows the importance of continuing such studies to better understand gastric defensive mechanisms in humans (26). Similar conclusions were obtained from the results of animal experiments, when we applied drugs (substances) acting on both efferent and afferent vagal nerves (19), and received a summary of both actions. The results of these observations led us to conclude that capsaicin-sensitive afferent nerves are the most important physiological regulators of gastric basal acid secretion and of chemical-induced gastric mucosal damage in human healthy subjects (26, 64, 65). The most important message of this study is that gastric capsaicin-sensitive afferentation has a permanent defensive role in the gastric mucosa against injury produced by different noxious agents. Consequently the modification of the function of capsaicin-sensitive afferent nerves offers new possibilities in the field of human medical therapy.

## **5. “Capsaicin and glucose absorption and utilization in healthy human subjects”**

### **5.1. Introduction**

A putative hepatic insulin-sensitive substance has been suggested to be of key importance in the regulation of peripheral insulin-sensitivity in healthy people and patients with diabetes (66). Oroszi et al. indicated that there was an interplay between the nitric oxide and calcitonin gene-related peptide produced by capsaicin. Oroszi et al. (67) and Sadri and Lauth (68) reported that the blockage of the hepatic nitric-oxide synthase causes insulin resistance.

Capsaicin activates TRPV1 expressed by a subgroup of primary afferent nociceptive neurons (69). The TRPV1 has been cloned (12) and is a cation channel. It is gated not only by capsaicin and some vanilloids but also by low pH, noxious heat and various pain-producing endogenous and exogenous chemicals. The potential role of vanilloid receptors in physiology and pathology was recently reviewed (70). The potential role(s) of capsaicin in glucose metabolism has been studied under different experimental circumstances in animal experiments (71, 72,73, 74, 75), but the experimental conditions of these animal studies differ significantly from each other and from those involving healthy human subjects. The main differences are: (1) higher doses of applied capsaicin; (2) significantly different time sequences between glucose administration and the hormonal response (insulin, glucagon release) and (3) different timing of capsaicin administration. The most significant difference between animal experiments and planned human observations is that orally administered glucose in human healthy subjects produces a well-defined sequence of events: glucose absorption from gastrointestinal tract → insulin release → glucagon release → glycogen mobilization from the liver.

The glucose tolerance test, carried out in healthy human subjects, offers an excellent scientific approach to gain insight into the physiological correlations between glucose absorption from the gastrointestinal tract (during the first 4 hour [h]), hormone (insulin and glucagon) release (from 1 to 2.5 h) and glycogen mobilization from the liver (from 2.5 to 3 h) without and with capsaicin (given in small doses), which stimulates the

capsaicin-sensitive afferent nerves in human healthy subjects (26). Earlier studies indicated a decreased basal gastric acid output (25) and an increase in gastric emptying (32) with capsaicin the ED<sub>50</sub> value being 400 µg capsaicin after oral administration in both cases.

In this study, the plasma levels of glucose, insulin, C-peptide and glucagon were measured every 15 minutes (min) to 4 h after oral administration of glucose without and with capsaicin in healthy human subjects.

## **5.2. Aim**

The aim of this study was to clear up the involvement of glucose absorption and hormone (insulin, C-peptide, glucagon) release in the neural regulation of hepatic insulin-sensitive substance, to prove the involvement of capsaicin-sensitive afferent nerves in glucose absorption and hormone regulation in human healthy subjects.

## **5.3. Materials and methods**

### *5.3.1. Clinical observations*

Fourteen healthy human subjects (aged 45±5, mean±S.E.D.) participated in this self-controlled study. Each healthy subject received 75 mg glucose orally (dissolved in 100 ml of water), without and with capsaicin (400 µg orally given). The plasma levels of glucose (Boehringer, Germany), insulin (µIU/ml) (Biochem Immunsystem), C-peptide and glucagon (pg/ml) (Byk-Sangtect Diagnostic GmbH) were measured in every 15 min for 4 h.

The observations were carried out according to Good Clinical Practice (GCP). The studies were approved by the Regional Ethics Committee of University of Pécs, Hungary. Written informed consent was obtained from all participants.

### *5.3.2. Chemical*

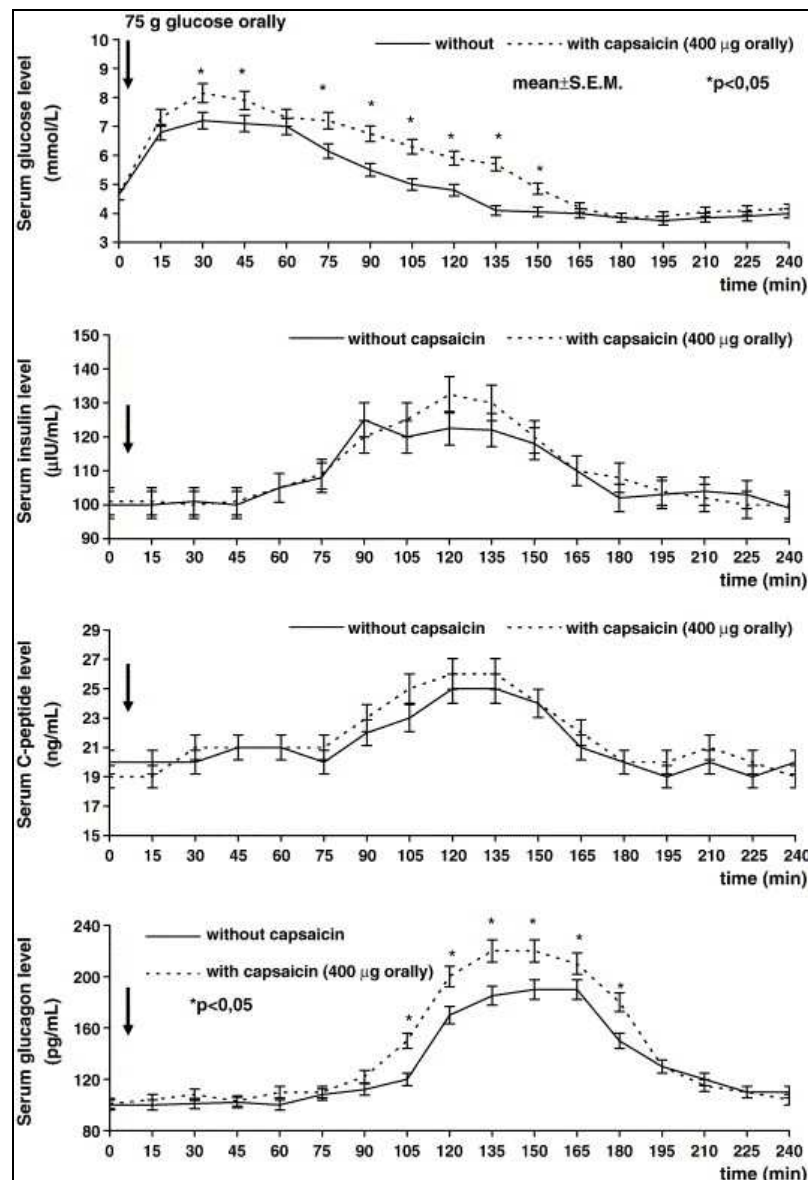
The pure capsaicin was obtained from Sigma-Aldrich, Budapest, Hungary.

### *5.3.3. Statistical analysis*

The results are expressed as means±S.E.M. The paired Student T-test was applied for statistical analysis of results for the same parameter. The results were taken to be significant if P≤0.05.

## 5.4. Results

The plasma levels of glucose increased significantly 30 to 150 min and the plasma level of glucagon increased from 90 to 180 min after capsaicin administration in human healthy subjects given 75 g glucose orally. The plasma levels of insulin and C peptide increased from 75 to 165 min after glucose administration; however, levels did not differ significantly without or with capsaicin (400  $\mu$ g orally) (Fig.5.1).



**Figure 5.1.** Changes in plasma levels of glucose, insulin, C-peptide and glucagon after oral administration of glucose (75 g in 100 ml water) in 14 healthy human subjects. Capsaicin (400  $\mu$ g) was orally given in gelatin capsule (Hunгарopharma, Budapest, Hungary). The plasma levels of glucose, insulin, C-peptide and glucagon were measured every 15 min for 4 h. The results are expressed as means  $\pm$  S.E.M.

## 5.5. Discussion

The response to glucose loading in healthy human subjects can be divided into three different periods on the basis of physiological regulatory events: (1) absorption (first period) (from 30 to 90 min); (2) insulin (and other hormones) release (second period) (from 60 to 150 min); (3) glycogen release (third period) by the liver (from 150 to 180 min). In the first period the plasma glucose level depends only on glucose absorption; in the second period the plasma level of glucose represents the equilibrium between the absorption and hormone release; and in the third period the glucose level represents the mobilization of glucose by the liver in healthy human subjects.

After the administration of capsaicin (400 µg orally) the plasma levels of both glucose and glucagon (the average value and as well as the peak of glucagon) increased significantly without there being any changes in plasma levels of insulin and C-peptide. By studying the time sequence of the changes in plasma levels of glucose, insulin, C-peptide and glucagon after glucose loading in healthy human subjects without and with capsaicin (400 µg), we observed the following: (1) The plasma levels of glucose (from 30 to 150 min) and glucagon (from 90 to 180 min) increased significantly after glucose plus capsaicin administration. (2) The plasma levels of insulin and C-peptide were increased on glucose loading in healthy human subjects from 90 to 165 min; however, no significant changes were observed between the subjects without and with capsaicin. (3) No significance in the timing of insulin and glucagon release was observed, which clearly excludes the existence of antagonism between the insulin and glucagon release (short time). (4) The plasma glucagon level was higher for longer than was the plasma insulin level. It should be noted that capsaicin increased glucagon levels only after glucose absorption had occurred. These results clearly indicate the complexity of glucagon release and the possible role of capsaicin-sensitive afferent nerves in its regulation.

We concluded from our present human observations that the increased release of glucagon is independent of insulin release after glucose loading that stimulation of capsaicin-sensitive afferent nerves by a low dose of capsaicin stimulates glucose absorption from the GI tract in healthy human subjects and promotes the mobilization of glycogen by stimulation of capsaicin-sensitive afferent nerves. Szolcsányi et al. (2004) showed the direct release of SS by the stimulation of capsaicin-sensitive afferent nerves

in rats. Capsaicin-induced glucagon synthesis is a part of the hormonal regulation of hepatic insulin-sensitive substance and is involved in the regulation of insulin resistance. The results clearly indicate that capsaicin-sensitive afferent nerves have a key role both in (increase due to a local increase in blood flow) gastrointestinal tract and in glucose utilization (increased glucagon release) by tissues in healthy human subjects. These effects of capsaicin occur at different times.

## 6. Brief general conclusion and near (present) future

The GI mucosal is an interactive surface inside of the body, which has different functions (for example absorption, immunodefence, digestion etc...). The integrity of the GI mucosa depends on the balance between aggressive (chemical agents, drugs and gastric acid) and defensive factors (gastric mucus, short/fast cell turn-over, faster gastric emptying, etc...). It is also well known that the gastric acid production has an important role in the development of gastric mucosal injury, because gastric ulcer could not be developed without gastric acid. This complex mechanism could be regulated by different neurohormonal agents (including the vagal nerve), and their roles were emphasized in the target of the development and treatment of gastric ulcer.

The key role of vagal nerve has been widely emphasized in the gastric acid production, and during the development of medicine the targets of anti-ulcer treatment changed/varied from anatomical structures (medical or surgical vagotomy) to molecular structures (H<sub>2</sub>R antagonists and of PPIs).

Interestingly, it was proved that the GI effects of the vagal nerve - as a part of a complex reflex arc - transmitted not only by the efferent but also by afferent fibers. Nowadays, the targets of the anti-ulcer treatment are on the efferent side of the vagal nerve, although the vagal nerve consists of 90% afferent fibers and 10% efferent fibers. This nine percent of the afferent fibers are sensitive for capsaicin so the capsaicin-sensitive afferent fibers and the efferent fibers (targets of the anti-ulcer treatment) are roughly the same amount (19).

Capsaicin is a member of the capsaicinoids (dihydrocapsaicin, nordihydrocapsaicin and other capsaicinoids) and they are the pungent ingredients of paprika or red pepper (65).

The effects of capsaicinoids/capsaicin acting on capsaicin-sensitive afferent nerves depend on the dose and the duration of the exposure and four different response stages [excitation (stage 1); sensory blocking effect (stage 2); long-term selective neurotoxin impairment (stage 3); irreversible cell destruction (stage 4)] could be separated according that (6; 11). In the first stage small doses of capsaicinoids stimulate these

nerve endings and active neurotransmitters are released from them. This mechanism could take a part in the gastroprotective effect.

Our work team takes part/is involved in the research for the gastroprotective effects of capsaicin in animal experiments from 1980 which results are summarized by Mózsik et al in 1997.

The human observations with capsaicin have been carried out at the first Department of Medicine, Medical and Health Center, University of Pécs from 1997. These studies were permitted by the Regional Ethical Committee of Pécs University, Hungary, and these observations were carried out according to Good Clinical Practice (GCP), respected the Helsinki Declaration.

The effect of capsaicin on capsaicin-sensitive afferent nerves depends on two different factors: the dose of the drug and the duration of the exposure (6).

The dose of capsaicin, which produces gastroprotective effect in healthy human subjects was determined by different, simply used methods (measurement of gastric BAO, gastric mucosal potential difference, or detection of the gastric microbleeding produced by IND with or without capsaicin) and the ED<sub>50</sub> of capsaicin on the gastric BAO was found to be 400 µg/person (26; 64).

To demonstrate the gastroprotective effect of capsaicin in healthy human subjects, when the effect of capsaicin was compared with other drugs acting on the muscarinic (atropine, pirenzepine), H<sub>2</sub>R (cimetidine, ranitidine, famotidine, nizatidine) or blocking of the proton pump (omeprazole, esomeprazole) and the pD<sub>2</sub> and the intrinsic activity (compared with the effects of atropine) were determinate (64).

The gastric mucosal damage and protection with capsaicin was studied with IND application (with and without capsaicin), which is a significant medical problem in the everyday medical treatment (as NSAIDs-induced gastrointestinal injuries, ulceration, bleeding, perforation). Because these studies were carried out in healthy human persons, therefore our attention turned to the directions of different GI disorders.

We planned to study the potential role(s) of the capsaicin-sensitive afferent fibers in different GI disorders (gastritis, ulcer, inflammation produced by different agents, like



*H. pylori*, precancerous states, cancers) in the proximal and distal parts of the GI tract (using classical endoscopic examinations and we obtained tissue samples by biopsy).

The role of this dissertation was to map the GI disorders in which the capsaicinoids (as TRPV1 receptor agonist) might influence its gastroprotective effect and to observe potential other pharmacological effects of the capsaicinoids in patients with different disorders. Of course, my investigations represent only a part of the pharmacological approach of capsaicin in the human beings, which were done our workteam. So the separation of these obtained results practically can be done.

The new results of our observations (in which I participated actively) are the followings:

1. The TRPV1 and SP and CGRP could be detected by immunohistochemical method in tissue samples of patients with different GI disorders. It means that the capsaicin-sensitive afferent nerves and neuropeptides released by them take part in the development of different human GI disorders.
2. The immunohistochemical distribution of TRPV1, CGRP and SP differs in the upper (gastric) and distal (colon) parts of the gastrointestinal tract (inflammation, hyperplasia, dysplasia, ulcer, polyps and cancers).
3. The involvement of capsaicin-sensitive afferent nerves in human chronic gastritis does not depend on the presence of *H. pylori* infection,
4. The capsaicin-sensitive afferentation has a permanent defensive role in the human gastric mucosa against injury produced by different noxious agents.
5. The increased activation (compared with histological healthy persons) of capsaicin-sensitive afferent nerves (by immunohistochemical distribution of TRPV1, CGRP and SP) after successfully *H. pylori* eradication therapy suggests that these nerves are involved in the healing mechanism of the gastric mucosa (that is a new capsaicin-sensitive afferent neural defensive pathway or mechanism in the treatment of *H. pylori*-induced chronic gastritis). This is an

internationally new observation in the field of treatment of chronic gastritis in patients.

6. The capsaicin-sensitive afferent nerves have a key role in glucose utilization (increased glucagon release) by tissues in healthy human subjects, indicating the participation of capsaicin action in the human carbohydrate metabolism.
7. Our results offered to suggest the participation of capsaicin-sensitive afferentation (including the TRPV1, released CGRP, SP) in the development and prevention of the different gastrointestinal disorders in patients.

Because the chronic gastric mucosal damage can be induced by different agents (especially by drugs and other causative agents such as *H. pylori*) and the direct orally application of capsaicin prevents the IND-induced mucosal damage and the increased activity of capsaicin-sensitive afferentation measured by immunohistochemical methods in *H. pylori* positive and negative chronic gastritis (which differs from the classical eradication treatment), these results together suggest the existence of the capsaicin-sensitive afferent dependent neural gastroprotective mechanism (64, 65), and it suggested that the capsaicin alone or in different combination can be used in the human medical therapy.

The results - as the gastroprotective effect of capsaicin - can be used in the treatment of *H. pylori* induced chronic gastritis, and the capsaicin (given in small doses) is able to prevent the NSAIDs-induced gastrointestinal mucosal damage - suggest that capsaicin might be able to prevent the gastric side effects of NSAIDs used as pain killers, antipyretic drugs or platelet aggregation inhibitors in patients with cardiac diseases, ischemic stroke, chronic joint diseases.

In the last years, the production of plant origin capsaicin alone or in combinations (capsaicin + acetylsalicylic acid, capsaicin + diclofenac and capsaicin + naproxen) was done in an innovative pharmacological research between the First Department of Medicine, Medical and Health Centre, University of Pécs versus the PannonPharma Pharmaceutical Ltd, Pécsvárad (76,78). Two from three human phase I. examinations were carried out in human healthy persons (capsaicin + ASA and capsaicin + diclofenac). The results of these finished human phase I. examinations indicated:

1. the capsaicin (orally given in doses of 400 and 800 µg) alone or in combinations could not be detected in the plasma of treated healthy subjects (which suggests that the capsaicin acts primarily locally in the pharmaceutical drug preparation or the pharmaceutical factory used excellent bioadhesive compound during the drug pharmaceutical production);
2. the different doses of capsaicin do not modify the pharmacokinetic parameters of the ASA and diclofenac;
3. capsaicin itself has no any effect on the platelet aggregation;
4. the ASA induced platelet aggregation remained unchanged.

So capsaicin might be able to prevent the gastric side effects of NSAIDs or ASA meanwhile it doesn't change the effects of them (NSAIDs and ASA) (77,78).

Finally, the results of basic animal examinations and human observations - carried out in First Department of Medicine, Medical and Health Centre from 1970 up to now - suggest that the gastrointestinal mucosal integrity could be reconstruct not only the "maximal inhibition" of the aggressive factors (H<sub>2</sub>R antagonists, PPIs and *H. pylori* eradication) but also the stimulation of the deffensive side (capsaicin→TRPV1).

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## 9. Publication related to the dissertation

### Published papers

Mózsik, Gy., Peidl, Zs., Szolcsányi, J., **Dömötör, A.**, Hideg, K., Szekeres, Gy., Karád, O., Hunyady, B.: Participation of vanilloid/capsaicin receptors, calcitonin gene-related peptide and substance P in gastric protection of omeprazole and omeprazole-like compounds. *Inflammopharmacology*. 13:139-159. (2005).

Independent citations: 1 All citations: 3

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Independent citations: 20 All citations: 22

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## 10. Publications not related to the dissertation

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## Abstracts

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