Immunolocalization and Expression of Kinin B1R and B2R Receptors in
Human Inflammatory Bowel Disease

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Running head: Kinin receptors in IBD.

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Abstract

Bradykinin is a mediator of inflammation, responsible for pain, vasodilation, and capillary permeability. Bradykinin receptor 1 (B1R) and bradykinin receptor 2 (B2R) are G-protein coupled receptors which mediate kinin effects. The latter is constitutive and rapidly desensitized; the former is induced by inflammatory cytokines and resistant to desensitization. The distribution of bradykinin receptors in human intestinal tissue was studied in patients with inflammatory bowel disease (IBD) – namely, ulcerative colitis (UC) and Crohn’s disease (CD). Both B2R and B1R proteins are expressed in the epithelial cells of normal and IBD intestines. B1R protein is visualized in macrophages at the center of granulomas in CD. B2R protein is normally present in the apices of enterocytes in the basal area and intracellularly in inflammatory tissue. In contrast, B1R protein is found in the basal area of enterocytes in normal intestine, but in the apical portion of enterocytes in inflamed tissue. B1R protein is significantly increased in both active UC and CD intestines compared to controls. In patients with active UC, B1R mRNA is significantly higher than B2R mRNA. However, in inactive UC patients, the B1R and B2R mRNA did not differ significantly. Thus, bradykinin receptors in IBD may reflect intestinal inflammation. Increased B1R gene and protein expression in active IBD provides a structural basis of the important role of bradykinin in chronic inflammation.

Keywords

Ulcerative colitis, Crohn’s disease, bradykinin, kinin receptors
Introduction

Two kallikreins, plasma and tissue; cleave kininogens to release kinins. Plasma kallikrein releases bradykinin (BK) from high molecular weight kininogen (HK), while tissue kallikrein liberates kallidin (Lys–BK) from low molecular weight kininogen (LK). There is little difference in the biological activity of BK and Lys-BK. Both are converted to desArg derivatives by carboxypeptidase N or M and become B1R receptor agonists (7). By opening the tight junctions between endothelial cells, BK can increase capillary permeability. BK stimulates sensory nerve endings, causing pain (22). BK has been demonstrated to stimulate synthesis of eicosanoids and nitric oxide production by both macrophages and endothelial cells.

In the past two decades, the role of plasma kallikrein in chronic inflammation has been well delineated (4). In our models of inflammatory arthritis and enterocolitis induced by proteoglycan-polysaccharide (PG-APS), activation of plasma kallikrein was documented in the genetically susceptible Lewis rat, but not in the resistant Buffalo rat (12). This activation is not specific for PG-APS since it can be induced by indomethicin in Lewis rats (23). A selective plasma kallikrein inhibitor has been shown to attenuate acute (19) and chronic (22) enterocolitis in the Lewis rat at low concentrations, which selectively inhibits plasma kallikrein. Activation of plasma kallikrein has been documented in patients with the active phase of UC (20). Agents that block BK receptors also modulate experimental inflammatory arthritis induced by PG-APS (23, 24). Recently, we have shown that experimental enterocolitis is 60% inhibited in rats deficient in HK and LK in a Lewis genetic background (26).

Less attention has been paid to the role of tissue kallikrein (TK) (6) in the pathogenesis of IBD. We have identified TK in goblet cells of the normal and inflamed rat colon (18), and we postulated that intestinal TK (ITK) was secreted at higher levels in inflammation. Recently we
localized human TK and the TK inhibitor, kallistatin (5), immunochemically in the intestinal mucosa of patients with IBD and normal subjects (21). The amount of immunoreactive TK and kallistatin was measured, and mRNA assessed for TK and kallistatin. Since the tissue level of TK and its inhibitor- kallistatin was decreased, we concluded that ITK was secreted during human IBD inflammation.

The aim of this study was to ascertain the concentrations of the B1R and B2R proteins as well as the expression of genes encoding of the B1R and B2R in inflamed tissues, and compared to controls. The alterations in both the distribution and levels of B1R and B2R proteins and mRNAs in patients with IBD compared to controls suggest a possible role of the kinins in the inflammatory process. Increased expression of B1R in active IBD provides a structural basis for the kinin function.

Materials and Methods

Patients and control subjects.

UC was diagnosed according to commonly accepted criteria, i.e. past and present clinical symptoms, sigmoidoscopy with biopsy, colonoscopy or barium enema. CD was diagnosed by clinical symptoms combined with barium contrast studies, endoscopy, and computer tomography. Clinical UC activity was assessed using a modification of Truelove and Witts UC activity index. Patients with a score of less than or equal to 2 were classified as mild colitis, those with a score from 3 to 6 were regarded as moderate colitis, and those with a score from 7 to 9 were considered as severe colitis (20). Disease activity of patients with CD was calculated according to the index of Best et al. (3). Patients with a score of 150 or greater were classified as
having active CD. Exclusion criteria included systemic infections and other serious cardiopulmonary, liver, or renal diseases as well as patients with imperiled fulminant colitis.

Blood was obtained from all patients and controls by venipuncture from the antecubital vein and anticoagulated in 3.8% sodium citrate (9:1, v:v). All samples were centrifuged at 3,000g for 15 min. Plasma samples were stored at -70°C until tested. Patients were studied in accordance with the protocol approved by the Institutional Committee on Human Subjects of the Medical University Hospitals. The patients gave written, informed consent.

**Immunolocalization of kinin receptors in human colonic tissue**

The specificity of rabbit anti–human polyclonal antibodies reactivity to B2 (mix AS287-83) as well B1 (mixAS434), used by us in all experiments has been reported. Antibodies were kindly donated by Werner Muller-Esterl, Ph.D., Institute of Biochemistry II, The University of Mainz, Germany. Eight samples of normal colonic tissue were obtained from patients undergoing partial colectomy for colon cancer, and inflammatory intestinal tissue samples were obtained after surgery from UC or CD patients. Tissue samples were fixed overnight in 10% neutral-buffered formaline (phosphate buffer). The samples were subsequently passed through graded alcohol solutions, processed three times in xylene, and finally embedded in paraffin blocks. Slices of 5 µm thickness were placed on Apes-coated slides, deparaffinized and rehydrated. To unmask the antigen, sections were boiled in 0.01 M citrate buffer (pH 6.0) in a microwave oven for 10 min at 800 W. Thereafter, sections were allowed to stand undisturbed for 20 min and washed twice (5 min each) in Tris buffered saline (TBS, 0.05 M, pH 7.4 and 0.85% NaCl). For quenching of endogenous peroxidase activity, tissue sections were blocked with 3% (v/v) H₂O₂ for 10 min. Prior to incubation with the primary antibody, the sections were
washed twice (5 min each) in TBS and were pretreated with normal goat serum for 30 min. The primary rabbit polyclonal antihuman antibodies to B2R and B1R, respectively, were added, and the sections were incubated overnight (4°C). This step was followed by incubation with biotinylated secondary antibody, and final visualization was achieved by using the ABC peroxidase kit (Vector Laboratories, Burlingame, CA) and freshly prepared 3,3’-diaminobenzidine tetrahydrochloride and hydrogen peroxide according to the protocol provided by the manufacturer. Normal rabbit serum instead of anti-B2R or anti-B2R antibodies was used as a control (9,21).

**Expression of B1R mRNA and B2R mRNA kinin receptors**

In the first series of the experiment, specimens of rectal biopsies were obtained from patients with UC (n=28) and non-inflamed controls (n=27) during colonoscopy. There were 12 UC men and 16 women, with a median age of 44 years. The control subjects (healthy subjects or individuals with gastrointestinal disturbances) were compatible with both subgroups (active and inactive UC patients) and matched for age and sex. Mucosal disease activity of UC patients was assessed as active (n=17 UC patients) or inactive (n=11 UC patients) using the modification of endoscopic index of Baron *et al.* (2), taking into consideration the mucosal vascular pattern, erythema, friability, granulation, erosions, and ulcerations. The patients were given conventional medical treatment. All UC patients received sulfasalazine or its derivatives. Five UC active patients and two UC inactive patients were taking moderate doses of corticosteroids and three of the UC patients (two in the active stage and one in the inactive stage) were on Azathioprine. The UC colonic extent of disease was variable; however, the specimens were taken from the rectum in all cases. The diagnosis of UC was confirmed histologically; rectal
biopsy specimens were stained with hematoxylin and eosin to determine the grade of histologic inflammation.

For molecular evaluation, rectal biopsy specimens of approximately 4 x 4 mm were removed by endoscopic forceps. The tissue samples were immediately frozen at -70ºC until use. The expression of B1 and B2 kinin receptor genes in biopsy specimens was evaluated as a number of mRNA copies by QRT-PCR using Sequence Detector ABI PRISM 7700 by Perkin Elmer. The single step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction was employed.

The numbers of mRNA molecules for B1 and B2 receptors in 1 µg total RNA were detected with ABI PRISM 7700 Sequence Detector, and with the primers and probe designed with the use of Primer Express 10 software. This was done on the basis of mRNA sequences obtained from the internet data bases of http://www.ebi.activation.uk and http:www.ncbi.nlm.nih.gov/PubMed in relation to the RT-PCR for β–actin, a marker of the number of copies for all genes studies in each reaction. We used complementary oligonucleotides: B2R: (FBR2: 5’CAC GGT GCT AGT CCT GGT TGT GCT-3’, RBR2: 5’-AGG TCC GCA GTG TGC CCA TG-3’ and SBR2: 5’-TCC AGC TGC CAG GAC GAG CGC ATC A-3’); B1R (FBR1: 5’-CTG CAC AGA GTG CTG CCG ACA TT-3; RBR1; 5’-ACA CCA GAT CAG AGG CTG CCA GG-3’; and SBR1; 5’-CCG GCG GCA ACT GAC GTG GCA GAA-3’). Probes used for the quantitative QRT-PCR analysis were coupled with fluorescent adducts: 5’-end with FAM (6-carboxyfluorescein), 3’-end with TAMRA (6-carboxy tetramethylrodamin). The number of mRNA molecules for B1 and B2 receptors in 1 µg total RNA were quantified using real-time PCR (TaqMan). First, a standard curve was constructed after amplification of the five concentrations of β–actin standards (26, 260, 2600, 5200, and
26000 copies) using β-actin Control Reagent Kit (Applied Biosystems). A standard curve was created for each separate experiment. As constructed, a 10-fold increase in the number of copies for β-actin mRNA contributes to the increase of 2-fold of the threshold cycle (Ct). The Ct is the number of cycles before the fluorescence emitted reaches a fixed limit. The $\log_{10}$ of the number of targets initially present in the sample is proportional to the Ct value and can be measured using the standard curve. In addition, for each sample, a number of mRNA β-actin (constitutive gene) copies was evaluated as an endogenous control of real-time PCR reaction. Finally, the number of B1 and B2 mRNA molecules present in each examined sample was calculated for 1 µg total RNA. Total RNA level was measured spectrophotometrically using the program GeneQuant DNA/RNA Calculator (Pharmacia INC).

**Kinin receptor levels in intestinal tissue samples**

In the second series of the investigation, colonic tissue specimens were taken from 15 UC active patients (10 men and 5 women, 50 years average age), 15 UC inactive patients (8 men and 7 women, 44 years age average), and 12 normal control subjects (6 men and 6 women, 49 years age average). These biopsy samples for immunohistochemical analysis were transferred directly to tubes containing 10% buffered formalin, fixed 4 h, and prepared as described above.

In the third series of the study, the colonic tissue samples were obtained from 10 active CD patients undergoing partial intestinal resection (4 men and 6 women, 39 years average) and 10 controls (normal colonic samples) matched according to sex and age. All UC patients were treated with Sulfasalazine, 5 active and 2 inactive with corticosteroids, and 4 active and 4 inactive with Azathioprine. Six CD patients had corticosteroids, with 3 of these additionally having Azathioprine and 2 with Azathioprine alone.
Tissue samples were prepared and stained with rabbit anti-human polyclonal antibodies for B2R and B1R, as described above. The number of positively stained epithelial cells for B2R and B1R in 8 different areas (173 x 130 microns) of each tissue sample using computer-assisted image analysis. In each enterocyte, the intensity of staining was measured as the optical density of the reaction product, as described in (13), with the program KS 100 VIDAS video image analyzer (Kontron Elektronic, Warsaw, Poland; served by IBAS 2.5 system and a Panasonic WV-CL700 digital camera) at a magnification of 25-fold (10,11,21). For each analyzed area, 173 x 130 microns average optical density per unit area was calculated. Finally, the arithmetic mean was calculated from 160 microscopic images for controls (20 tissue samples x 8 areas) and 120 for UC and 120 for CD.

**Statistical analysis**

The significance of differences between the means of each group was determined by the Students’ \( t \) test. In the immunochemical data, the means were compared using the Kolmogorov-Smirnov test and the Mann–Whitney U-test. Because the mRNA concentration was distributed logarithmically, we evaluated the significance of the differences between the controls, active UC patients, and inactive UC patients for the measurement for B2R mRNA and B1R mRNA expression using the logarithmic values of each parameter. The means were compared using the Mann-Whitney U test. All values were expressed as mean ± SEM.
Results

**Immunochemical localization kinin B2R and B1R receptors**

The specificity of rabbit polyclonal antibodies to human B2 and B1 kinin receptors, respectively, used by us has been previously reported. We visualized both B1 and B2 kinin receptors in normal as well as inflammatory human colon and ileum. Figure 1A illustrates localization of immunoreactive B2R in the epithelial cells, mostly in the apices of enterocytes of the normal human colon.

![Figure 1A](image-url)
Figure 1B demonstrates B2R mainly intracellularly and in the basal part of enterocytes in inflammatory ulcerative colitis tissue.

In Figure 1C, staining for B2R is shown in the endothelial cells of blood vessel walls in the colonic submucosa.
Figure 1D shows B1R localization in the epithelial cells of normal colon, mainly immunostained in the basal part of enterocytes (in striking contrast to B2R localized mainly in the apical part).

Figure 1E presents the positive reaction for B1R in the gladularis mucosa of UC-inflamed colon, the positive staining in the apical part of enterocytes as well as intracellularly.
Figure 1F shows the B1 receptor in the apices of enterocytes in CD inflammatory tissue as well as in plasmocytes.

Figure 1G presents the positive staining for B1R in the nerve of the colonic submucosa, whereas
Figure 1H presents the immunoreactive staining for B1R in macrophages inside granulomas of CD intestine. However, specific antibodies against the B2 receptor did not show a positive reaction in granulomas (data not shown). Intestinal sections treated with non-immune rabbit serum instead of B1R or B2R antibodies were always unstained (not shown).

Quantitation of kinin receptors in intestinal samples

Positive immunohistochemical reaction for both B1R and B2R were observed in a vast majority of enterocytes in both normal and inflamed colon. To compare the level of both B1R and B2R in normal and inflamed intestinal tissue, we quantified the intensity of staining in mucosal tissue samples using image analysis. The mean level of B1R in the apical part of enterocytes in active UC (124.5 ± 11 SE) as well as inactive UC (107.9 ± 7.7) was significantly (p <0.0001) higher as compared with non-inflamed control (50.5 ± 1.9) (Table I). However, there was no statistical difference in mean level of B1R in the apices of enterocytes between the UC active and UC inactive patients. In contrast, image analysis revealed the significant decrease (p <0.0001) of B1R in basal part of enterocytes in UC active inflammatory specimens (69.8 ±
as well as UC inactive specimens (62.4 ± 3.2) as compared with normal controls (106.9 ± 9). Finally, the total level of B1R was significantly higher (p <0.003) in enterocytes of patients with active phase of UC (110.8 ± 2.5) as compared with controls (87.9 ± 2.7) (Table II).

A different pattern was observed in B2R distribution and levels in enterocytes. The mean level of B2R in the apical part of enterocytes was significantly lower in UC active patients (123.6 ± 12, p <0.01) and UC inactive patients (119.3 ± 10.7, p <0.003), as compared with controls (134.9 ± 14). In the basal part of enterocytes, the mean level of B2R was significantly increased (p < 0.02) in patients with active UC (82.05 ± 0.7) but not in patients with inactive UC (65.9 ± 6.0), as compared with controls (64.3 ± 4.6). Finally, no statistical difference was observed when comparing the total level of B2R in enterocytes of UC inflammatory groups and that of normal controls (Table II).

Similarly, the B1R as well as the B2R level was calculated in tissue samples of active CD patients (Table IIA). The mean level of B1R was found to be significantly higher (p <0.001) in the apical part (130.6) as well as the basal part: 101.3 ± 13.8 of enterocytes in CD patients as compared with controls, 107.4 and 101.3, respectively. In contrast, the mean level of the B2 receptor significantly decreased (p<0.001) in the apical part of CD enterocytes (143.9 ± 25.0) as compared with controls (165.4 ± 22), whereas in the basal part of CD enterocytes, the B2 level was higher (128.2 ± 20, p <0.001) than in controls (109.2 ± 18.6).

**B1R and B2R receptor gene expression**

In patients with the active phase of UC, mRNA expression for B1 receptor (887 ± 292 SE, the number of copies mRNA/ug RNA) was significantly higher (p <0.02) as compared with the mRNA level for B2 receptor (221 ± 52). However, in UC inactive patients, the B1 and B2
receptor expression level did not differ significantly. The mean level of copies for B1 in UC active patients (887 ± 292) as well as in UC inactive patients (1660 ± 658) was significantly lower as compared with controls (5420 ± 1316). Similarly, B2 mRNA levels were significantly lower (p <0.01) in active UC (221 ± 54) mRNA copies/µg RNA) and to UC inactive patients (934 ± 431), as compared with the non-inflammatory controls (5500 ± 1795). Finally, the B1/B2 expression ratio was calculated in normal and UC inflammatory tissue (Table III). The B1/B2 ratio was 0.9 in normal tissue, increased in UC inactive phase at 1.8, and found to be the highest in the UC active phase at 4.03.

Discussion

Recently, we have focused our attention on the tissue kallikrein-kinin system in IBD. To study the localization of TK and its naturally occurring serine protein inhibitor (SERPIN), kallistatin, in human IBD, we first turned to a rat model of PG-APS stimulated chronic inflammation closely resembling CD (18). We showed that the normal location of TK was the goblet cells and that substantial amounts of ITK were present in the macrophages of the granuloma found in the submucosa, suggesting that ITK is present at the site of inflammation. In addition, ITK was found to be lower in the supernatant from in vitro cultures of inflamed intestine (17). This combination suggested secretion of ITK during inflammation.

We postulated that if human IBD resembled the rat, many of the same findings would occur in human IBD as in experimental rat enterocolitis. We extended the findings and enhanced them by characterizing the cellular localization, concentration, and mRNA of kallistatin in addition to ITK. The demonstration that ITK is in the goblet cells of normal and inflamed
human colon and macrophages of intestine for IBD (21) is in agreement with our previous findings in rats (18).

A new observation was that plasma cells on the border of the granuloma contain large amounts of TK. This observation emphasizes the close relationship between the immune responses important in IBD and inflammatory mediators, including the kallikrein-kinin system. We again find that TK levels were significantly decreased in inflamed intestinal tissue, and we observed the decrease of intestinal kallistatin concentration in IBD using two different methods. In normal colon, kallistatin is physically separate from TK, but, during inflammation, the inhibitor is available to neutralize TK. This process is reflected in the plasma where kallistatin is decreased in both UC and CD relative to normal subjects, similar to the findings in the rat model. Kininogen has been demonstrated in both normal and inflamed human colon; thus, TK can generate BK.

In the present study, we investigated for the first time the expression of genes encoding kinin receptors and the precise localization of these two proteins in normal and human inflamed intestinal tissue. Using specific immunostaining and image analysis for quantification, we observed in the normal intestine not only constitutive B2R but also inducible B1R, which suggests that both receptors may play a role in intestinal physiology. In general, in the normal condition B1R is present in most tissue only in minimal amounts but is induced during the inflammatory process upon exposure to proinflammatory cytokines, especially IL-1β and endotoxins. Recently Sawant et al. (16) assessed immunoreactive kinin receptors in normal and inflamed gastric mucosa. In contrast to our data, they observed only B2R, but not B1, in unchanged tissue, whereas B1R protein was upregulated during gastritis. However, in normal rectum (in contrast to healthy gastric mucosa), a moderately increased leukocyte infiltration may
reflect the physiologic response to normal microbial stimuli. Thus, normal gut is not inflamed; but, in the presence of inflammatory cells such as leukocytes, this also can be considered normal in rectal tissue.

In inflamed active IBD tissue, we documented a significantly higher B1R protein level as compared with the controls, in all probability stimulated by endotoxin (LPS), which is detectable in almost all active IBD patients and, in turn, may mediate endothelial perturbation which results in leukocyte stimulation with cytokine release. We also observed a co-localization of TK and kallistatin as well as kinin receptor proteins in the endothelium of intestinal vessels. In macrophages forming granulomas, we found positive staining again for TK kallistatin and the B1 receptor, but not the B2 receptor, indicating the significance of the B1R-related pathway in Crohn’s inflammation.

Despite a moderate increase of B1R protein level in active inflammatory tissue, the mRNA level for both B1R and B2R were found to be reduced in inflamed colon as compared with the normal control. The discrepancy between the receptor protein concentration and its mRNA level is difficult to explain. In UC, local homeostasis is disrupted, leading to active inflammation to epithelial cell loss and ulcer formation. However, in the present study we calculated gene expression for kinin receptors in relation to β-actin gene expression. The β-actin gene is unchanged in inflammation and thus serves as an endogenous denominator of gene expression from the same cell. Thus, the decrease of both B1 and B2 kinin receptor encoding genes is due to an alteration of transcription rather than an effect of cell loss. It is well recognized that exposure to endotoxins may cause, in vitro and in vivo, a refractory state to the synthesis of cytokines as well as nitric oxide. Alternatively, an abundance of protein for both kinin receptors in the vast majority of enterocytes may feedback to inhibit new kinin receptor
synthesis and lower mRNA level in inflamed tissue. In addition, the B2R receptor may be recycled several times in the same enterocytes after internalization (1). This process is supported by the appearance of B2R intracellularly in some enterocytes in UC intestine (17). In contrast, the B1 receptor is thought not to be internalized, but rather redistributed, to caveolae-related lipid rafts in the cell membrane (14). Regardless of transcriptional and/or translational modification, the most striking of our results is the increase in the ratio of B1R to B2R gene expression in relation to the degree of intestinal inflammation. Thus, the increase of the B1 receptor gene expression (relative to B2R gene expression) is concomitant with an increase of B1R protein concentration in UC active intestine, indicating that the B1R receptor is a major structural background for kinin function in IBD. These observations provide insight in the pathogenesis of IBD and suggest that selective B1R inhibitors may have potential in a therapeutic trial. However, we have found (24) that B1 receptor antagonists in rat enterocolitis appear to aggravate the inflammation, suggesting that further studies are needed.

What is the role of kinins in the inflamed colon? BK not only has many proinflammatory actions, as indicated earlier, but also can stimulate release of mediators for endothelial, epithelial, and white blood cells, such as thromboxanes (12), NO (8), and cytokines, known to be important in IBD. Receptors for these reactions are present in the human intestinal epithelium and, thus, BK can initiate these inflammatory reactions in the inflamed ileum and colon.

Acknowledgment

We thank Werner Muller-Esterl, Ph.D., Institute of Biochemistry II, The University of Mainz, Germany, for the donation of antibodies against B2 and B1 kinin receptors.
Grants

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References


Figure legends

Figure 1. Immunolocalization of kinin B1 and B2 receptors in human normal and IBD colonic tissue. 1A) B2 receptor in normal colon, more intensity staining in apical part of epithelial cells; x 280. 1B) B2 receptor in enterocytes of UC colon. Specific staining mainly in the basal part of enterocytes; x 360. 1C) B2 in vessel endothelium of Crohn’s disease colon; x 280. 1D) B1 receptor in epithelial cells of normal colon x 280. 1E) B1 receptors in enterocytes of UC colon x 360. 1F) B1 in enterocytes of Crohn's disease colon. Positive reaction mainly in the apical part of the cells; x 560. 1G) Specific staining for B1 in nerves localized in UC colon; x 280. 1H) B1 receptor in macrophages within granuloma x 200.
Table I. Level B1R and B2R with Enterocytes

<table>
<thead>
<tr>
<th>Optical density units</th>
<th>B1R mean ± SE</th>
<th>B2R mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apical part</td>
<td>50.5 ± 1.9</td>
<td>139.4 ± 14.0</td>
</tr>
<tr>
<td>Basal part</td>
<td>106.7 ± 9.0</td>
<td>64.3 ± 4.64</td>
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<tr>
<td>Inactive UC</td>
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</tr>
<tr>
<td>Apical part</td>
<td>107.9 ± 7.7*</td>
<td>119.3 ± 10.7*</td>
</tr>
<tr>
<td>Basal part</td>
<td>62.4 ± 3.2*</td>
<td>65.9 ± 6.0</td>
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<tr>
<td>Active UC</td>
<td></td>
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</tr>
<tr>
<td>Apical part</td>
<td>124.5 ± 11.0*</td>
<td>123.7 ± 12.0*</td>
</tr>
<tr>
<td>Basal part</td>
<td>69.8 ± 3.2*+</td>
<td>82.0 ± 5.1*+</td>
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* Statistical significance, active UC as well as inactive UC vs. controls, p< 0.05

+ Statistical significance, inactive UC vs. active UC, p< 0.05
Table II.  Total Level of B1 and B2 Receptors in Enterocytes

<table>
<thead>
<tr>
<th></th>
<th>Receptor B1 optical density units mean ± SE</th>
<th>Receptor B2 optical density units mean ± SE</th>
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<tr>
<td>Controls</td>
<td>87.9 ± 27.6</td>
<td>117.6 ± 494</td>
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<tr>
<td>Inactive UC</td>
<td>96.4 ± 21.2</td>
<td>104.1 ± 36.5</td>
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<tr>
<td>Active UC</td>
<td>110.9 ± 25.6 *</td>
<td>113.1 ± 21.1</td>
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* Statistical significance between active UC and controls, p<0.0271
Table II A. Level of B1 and B2 Receptor Proteins (optical density units mean ± SE) in Crohn’s Intestinal Tissue and Controls

<table>
<thead>
<tr>
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<th>Control – Apex</th>
<th>101.5 ± 12.3</th>
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<tr>
<td>B1</td>
<td>Crohn’s – Apex</td>
<td>130.6 ± 18.0, p &lt; 0.001</td>
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<tr>
<td>B1</td>
<td>Control – Basal</td>
<td>111.6 ± 20.1</td>
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<tr>
<td>B1</td>
<td>Crohn’s – Basal</td>
<td>107.3 ± 13.8</td>
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<tr>
<td>B2</td>
<td>Control – Apex</td>
<td>165.5 ± 22.0</td>
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<tr>
<td>B2</td>
<td>Crohn’s – Apex</td>
<td>143.9 ± 25.9, p &lt; 0.01</td>
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<tr>
<td>B2</td>
<td>Control – Basal</td>
<td>109.2 ± 18.6</td>
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<td>Crohn’s – Basal</td>
<td>128.2 ± 20.7, p &lt; 0.01</td>
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Table III: Expression of Gene Encoding B1 and B2 Receptors

<table>
<thead>
<tr>
<th>Number of mRNA copies/µg RNA</th>
<th>Control group</th>
<th>Inactive UC</th>
<th>Active UC</th>
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<tr>
<td>Ratio B1/B2</td>
<td>0.9</td>
<td>1.8</td>
<td>4.03</td>
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