Immunolocalization and expression of kinin B$_1$R and B$_2$R receptors in human inflammatory bowel disease

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Bradykinin is a mediator of inflammation, responsible for pain, vasodilatation, and capillary permeability. Bradykinin receptor 1 (B$_1$R) and bradykinin receptor 2 (B$_2$R) are G protein-coupled receptors that 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 B$_1$R and B$_2$R proteins are expressed in the epithelial cells of normal and IBD intestines. B$_1$R protein is visualized in macrophages at the center of granulomas in CD. B$_2$R protein is normally present in the apexes of enterocytes in the basal area and intracellularly in inflammatory tissue. In contrast, B$_2$R protein is found in the basal area of enterocytes in normal intestine but in the apical portion of enterocytes in inflamed tissue. B$_1$R protein is significantly increased in both active UC and CD intestines compared with controls. In patients with active UC, B$_1$R mRNA is significantly higher than B$_2$R mRNA. However, in inactive UC patients, the B$_1$R and B$_2$R mRNA did not differ significantly. Thus bradykinin receptors in IBD may reflect intestinal inflammation. Increased B$_1$R gene and protein expression in active IBD provides a structural basis of the important role of bradykinin in chronic inflammation.

ulcerative colitis; Crohn’s disease; bradykinin

TWO KALLIKREINS (plasma and tissue) cleave kininogens to release kinins. Plasma kallikrein releases bradykinin (BK) from high-molecular-weight kininogen (HK), whereas 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 bradykinin receptor 1 (B$_1$R) 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 indomethacin 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 ulcerative colitis (UC; see Ref. 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 (8a).

Less attention has been paid to the role of tissue kallikrein (TK; see Ref. 6) in the pathogenesis of inflammatory bowel disease (IBD). We have identified TK in goblet cells of the normal and inflamed rat colon (18), and we postulated that intestinal tissue kallikrein (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 was assessed for TK and kallistatin in intestinal tissue kallikrein (ITK) was secreted at higher levels in inflammation. Increased B$_1$R gene and protein expression in active IBD provides a structural basis of the important role of bradykinin in chronic inflammation.

The aim of this study was to ascertain the concentrations of the B$_1$R and bradykinin receptor 2 (B$_2$R) proteins and the expression of genes encoding the B$_1$R and B$_2$R in inflamed tissues compared with controls. The alterations in both the distribution and levels of B$_1$R and B$_2$R proteins and mRNAs in patients with IBD compared with controls suggest a possible role of the kinins in the inflammatory process. Increased expression of B$_1$R in active IBD provides a structural basis for 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. Crohn’s disease (CD) was diagnosed by clinical symptoms combined with barium contrast studies, endoscopy, and computer tomography. Clin-
The specimens were taken from the rectum in all cases. The diagnosis of inflammatory bowel disease was based on clinical examination, laboratory investigations, and endoscopy. All UC patients received sulfasalazine or its active metabolite, mesalamine, and were also treated with corticosteroids and azathioprine. Six CD patients had corticosteroids, with three of them also receiving azathioprine. Two CD patients were treated with sulfasalazine, five active and two inactive UC patients were treated with sulfasalazine, five active and two inactive UC patients were treated with mesalamine, and four active and four inactive UC patients were treated with azathioprine. Six CD patients had corticosteroids, with three of these additionally having azathioprine and two 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 eight normal control subjects (6 men and 6 women, 49 yr age average) was measured using stereophotometrically using the program GeneQuant DNA/RNA Calculator (Pharmacia).
VIDAS video image analyzer served by IBAS 2.5 system and a Panasonic WV-CL700 digital camera (Kontron Elektronic, Warsaw, Poland) at a magnification of 25-fold (10, 11, 21). For each analyzed area, 173 × 130 μm average optical density per unit area was calculated. Finally, the arithmetic mean was calculated from 160 microscopic images for controls (20 tissue samples × 8 areas), 120 for UC, and 120 for CD.

Statistical analysis. The significance of differences between the means of each group was determined by the Student’s t-test. In the immunohistochemical 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 means ± SE.

RESULTS

Immunohistochemical localization of kinin B2R and B1R. The specificity of rabbit polyclonal antibodies to human B2 and B1 kinin receptors used by us has been reported previously (1). We visualized both B1 and B2 kinin receptors in normal and inflammatory human colon and ileum. Figure 1A shows local-
G364 KININ RECEPTORS IN IBD

Table 1. Level of B1R and B2R with enterocytes

<table>
<thead>
<tr>
<th></th>
<th>B1R</th>
<th>B2R</th>
</tr>
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<tbody>
<tr>
<td>Controls</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>
</tr>
<tr>
<td>Inactive UC</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>
</tr>
<tr>
<td>Active UC</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>
</tr>
</tbody>
</table>

Values are means ± SE; units are optical density (OD). B1R, bradykinin receptor 1; B2R, bradykinin receptor 2; UC, ulcerative colitis. P < 0.05, statistical significance, active UC and *inactive UC vs. controls. †Inactive UC vs. active UC.

Table 2. Total level of B1R and B2R in enterocytes

<table>
<thead>
<tr>
<th></th>
<th>B1R</th>
<th>B2R</th>
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<tbody>
<tr>
<td>Controls</td>
<td>87.9 ± 27.6</td>
<td>117.6 ± 494</td>
</tr>
<tr>
<td>Inactive UC</td>
<td>96.4 ± 21.2</td>
<td>104.1 ± 36.5</td>
</tr>
<tr>
<td>Active UC</td>
<td>110.9 ± 25.6*</td>
<td>113.1 ± 21.1</td>
</tr>
</tbody>
</table>

Values are means ± SE; units are OD. *Statistical significance between active UC and controls, P ≤ 0.0271.

Table 3. Level of B1R and B2R proteins in Crohn’s intestinal tissue and controls

<table>
<thead>
<tr>
<th></th>
<th>B1R</th>
<th>B2R</th>
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<tbody>
<tr>
<td>Control-apex</td>
<td>101.5 ± 12.3</td>
<td></td>
</tr>
<tr>
<td>Crohn’s-apex</td>
<td>130.6 ± 18.0*</td>
<td></td>
</tr>
<tr>
<td>Control-basal</td>
<td>111.6 ± 20.1</td>
<td></td>
</tr>
<tr>
<td>Crohn’s-basal</td>
<td>107.3 ± 13.8</td>
<td></td>
</tr>
<tr>
<td>Control-apex</td>
<td>165.5 ± 22.0</td>
<td></td>
</tr>
<tr>
<td>Crohn’s-apex</td>
<td>143.9 ± 25.9†</td>
<td></td>
</tr>
<tr>
<td>Control-basal</td>
<td>109.2 ± 18.6</td>
<td></td>
</tr>
<tr>
<td>Crohn’s-basal</td>
<td>128.2 ± 20.7†</td>
<td></td>
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</tbody>
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Values are means ± SE; units are OD. *P < 0.001. †P < 0.01.

DISCUSSION

Recently, we have focused our attention on the TK-kinin system in IBD. To study the localization of TK and its...
In inflamed active IBD tissue, we documented a significantly higher B1R protein level compared with the controls, in all probability stimulated by endotoxin, which is detectable in almost all active IBD patients and, in turn, may mediate endothelial perturbation that results in leukocyte stimulation with cytokine release. We also observed a colocalization 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 B1R, but not the B2R, 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 was found to be reduced in inflamed colon 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 the result of 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 and nitric oxide. Alternatively, an abundance of protein for both kinin receptors in the vast majority of enterocytes may feed back to inhibit new kinin receptor synthesis and lower mRNA level in inflamed tissue. In addition, the B2R may be recycled several times in the same enterocytes after internalization (1a). This process is supported by the appearance of B2R intracellularly in some enterocytes in UC intestine (17). In contrast, the B1R is thought to not 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 B1R gene expression (relative to B2R gene expression) is concomitant with an increase of B1R protein concentration in UC active intestine, indicating that B1R 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 B1R 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), nitric oxide (8), and cytokines, known to be important in IBD. Receptors for these reactions are present in the human intestinal epithelium; thus, BK can initiate these inflammatory reactions in the inflamed ileum and colon.

ACKNOWLEDGMENTS

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

Table 4. Expression of gene encoding B1R and B2R

<table>
<thead>
<tr>
<th>Control Group</th>
<th>Inactive UC</th>
<th>Active UC</th>
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<tbody>
<tr>
<td>Ratio B1/B2</td>
<td>0.9</td>
<td>1.8</td>
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</table>

Units are no. of mRNA copies/μg RNA.
GRANTS

This work was supported by KBN Grants 2 PO5B 140 26 (A. Standnicki) and 4 PO5B 1R5618 (A. Standnicki), in part, by a grant from the Crohn’s & Colitis Foundation of America (R. W. Colman), and, in part, by Grant IBD-0080 from the Broad Foundation for Medical Research (R. W. Colman).

REFERENCES


