Butyrate Hastens Restoration of Barrier Function after Thermal and Detergent Injury to Rat Distal Colon in Vitro

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Background: Epithelial migration restores barrier function after superficial injury to any mucosa. The present study aimed to determine whether butyrate, important to colonic epithelial physiology in diverse ways, influences restoration of barrier function in the injured rat colon.

Methods: Rat distal colon was transiently exposed in vitro to heat (55°C for 10 sec) or to detergent (deoxycholic acid, 7.5 mM, for 15 min), and tissue damage was verified histologically. Epithelial barrier function was assessed, in colon tissue mounted in Ussing chambers, by measuring electric resistance and passive serosa-to-mucosa fluxes of 22Na and of 14C PEG 4000 under voltage clamp conditions. Studies were done in the absence and presence of 25 mM butyrate in the bathing solutions.

Results: Heat exposure induced superficial epithelial damage, and the electric resistance decreased significantly. This was accompanied by increase in flux of 14C PEG and increased passive flux of 22Na. Electric resistance was significantly higher, and PEG flux significantly lower, in tissues bathed with butyrate. Exposure to deoxycholic acid also induced superficial epithelial damage, reduced tissue electric resistance, and increased passive flux of Na and PEG. Electric resistance was significantly higher, and PEG flux significantly lower, in injured tissues bathed in butyrate, than in injured tissues bathed in butyrate-free solution. The effect of butyrate on restoration of electric resistance towards normal was seen in colon both from adult rats and from younger rats that were 2 or 6 weeks old.

Conclusions: Butyrate enhanced restoration of mucosal barrier function in rat distal colon in response to heat and detergent injury.

Key words: Butyrate; epithelial restitution; mucosal barrier; permeability

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The colonic mucosa is an important barrier separating potentially noxious elements in the bowel lumen from the internal milieu of the body. The barrier, which is a function of colonic epithelial cells and intervening tight junctions (1), prevents pro-inflammatory molecules in the bowel lumen from gaining access to the lamina propria. Any disturbance of barrier function, irrespective of the underlying cause, is followed by mucosal inflammation (2). In health, the paracellular pathway is the main pathway of passive absorption across the mucosa. In disease, increased mucosal permeability correlates with the presence of intestinal mucosal inflammation. Mucosal permeability to ethylenediaminetetraacetic acid and to polyethylene glycol 600 are increased in active ulcerative colitis (3, 4) and in Crohn disease patients and their relatives (5). Similarly, colitis in experimental animals is associated with an increased permeability to polyethylene glycol 4000 (6). Impaired mucosal barrier function is equally important in infectious diarrhoea, and the increased permeability may contribute to diarrhoea. Defects of barrier function in cultured intestinal epithelial cell monolayers have been shown to result from infection with bacteria and parasites and from exposure to bacterial toxins (7–9).

Superficial injury to the colonic mucosa has been used as a technique to study breakdown and repair of the epithelial barrier. As an immediate response to breakdown of the barrier, actin arcs and rings form in an effort to approximate the edges of the wounded area (10). Simultaneously, adjacent epithelial cells extend lamellipodia, leading to flattening and migration of the crypt epithelium to cover gaps in the surface (11). This phenomenon, called epithelial restitution, occurs within a few hours and is followed hours later by increased epithelial cell proliferation and re-epithelialization (12). The alteration in barrier function can be assessed functionally by studying whole intestinal wall mounted in flux chambers and measuring electric resistance in addition to passive transport of molecules of different sizes (13–15).

Butyrate, a short-chain fatty acid produced in the colon by bacterial fermentation of unabsorbed carbohydrate, plays an important physiologic role in the maintenance of the health and integrity of the colonic epithelium (16). In cultured colonic cell lines not exposed to deleterious influences,
butyrate reduces paracellular permeability, as indicated by a decrease in the tissue electric conductance and decreased permeation of mannitol (17). In colonic cell monolayers that have been wounded by physical means, butyrate stimulates epithelial migration to cover the wound (18).

The present study was designed to investigate the role of butyrate in the regulation of epithelial restitution in rat distal colon mucosa in response to two different kinds of injury—namely, thermal and detergent injury.

Materials and Methods

Solutions used

The following solutions were used: butyrate-free Ringer solution contained: 115 mmol/l NaCl, 25 mmol/l NaHCO₃, 2.4 mmol/l K₂HPO₄, 0.4 mmol/l KH₂PO₄, 1.2 mmol/l MgCl₂, 1.2 mmol/l CaCl₂, and 10 mmol/l glucose; pH 7.4; butyrate-containing Ringer solution contained 90 mmol/l NaCl, 25 mmol/l NaHCO₃, 2.4 mmol/l K₂HPO₄, 0.4 mmol/l KH₂PO₄, 1.2 mmol/l MgCl₂, 1.2 mmol/l CaCl₂, 25 mmol/l Na butyrate, and 10 mmol/l glucose; pH 7.4.

Tissue preparation and manipulations

The colon was removed from adult Wistar rats (200–250 g) after ether anaesthesia, rinsed with saline, and opened longitudinally along the mesenteric line. Square pieces of distal colon, with serosa and muscularis intact, were used for experiments in Ussing chambers. Tissue was also obtained from 6-week-old and 2-week-old rats but was only used for measurements of electric resistance.

To induce thermal injury, the colonic mucosa was immersed for 10 sec in oxygenated butyrate-free solution at 55 °C. Preliminary studies indicated that this degree of heat exposure provided a reproducible decrease in tissue electric resistance.

Detergent injury was induced, after the tissue had been mounted in Ussing chambers, by the addition of 7.5 mM deoxycholic acid (final concentration) to butyrate-free solution in the mucosal reservoir of the Ussing chamber setup. The deoxycholic acid-containing solution was removed after 15 min and replaced with either butyrate-free or butyrate-containing solution (without deoxycholic acid).

Electric and permeability measurement

All experiments were performed using distal colon tissue mounted in Ussing chamber setups (World Precision Instruments, USA). The chambers used had an exposed surface area of 1.13 cm². The mucosal and serosal bathing solutions were identical and composed of either butyrate-free or butyrate-containing solution. Butyrate was added to both mucosal and serosal solutions, to eliminate chemical gradients that may have had additional effects on transport. The solutions in the reservoirs were oxygenated and maintained at a temperature of 37 °C. They were connected via agar bridges to calomel electrodes and Ag/AgCl electrodes, which in turn were connected to DVC-1000 (World Precision Instruments) voltage clamps.

Transmural potential difference (PD) was measured at 15-min intervals. Otherwise, the spontaneous PD was short-circuited so that the tissue was clamped at zero transmural voltage. Base-line short-circuit current (Isc) and conductance values were recorded after the tissues had been allowed to equilibrate for 20 min. The electric resistance of the tissue was determined from the PD and the imposed current by applying Ohm’s law. This value provides an indication of the barrier properties of the epithelium to passive movements of ions across the tissue (1). To correspond with the flux results, the 0-time electric resistance measurements recorded actually refer to measurements made 20 min after tissue was mounted in the Ussing chambers.

The permeability of the tissues was assessed by measuring the unidirectional serosal-to-mucosal flux of the non-absorb-
able probe $^{14}$C-PEG 4000 (polyethylene glycol of average molecular weight 4000) and of $^{22}$Na (Amersham, UK). Flux experiments were performed under short-circuited conditions. After a stable Isc developed, $^{14}$C-PEG (29.4 $\mu$M/l) and $^{22}$Na (95 eq/l) were added to the serosal reservoir. After 20 min of equilibration, a 1-ml sample of the mucosal solution was removed for analysis and replaced with the appropriate solution. Further samples were taken from the mucosal reservoir at 1, 2, 3, and 4 h.

$^{14}$C and $^{22}$Na were measured with beta and gamma counters (Rackbeta and Compugamma, LKB Wallac, Sweden). Fluxes of Na and PEG were calculated in accordance with the following formula, and expressed as $\text{mol/cm}^2/\text{h}$:

$$\frac{(\text{cpm}_1 \text{mol} - \text{cpm}_2 \text{mol}) \times \text{concentration}}{\text{Surface area} \times \text{cpm}(H)}$$

$\text{cpm}_1$ and $\text{cpm}_2$ are counts per minute of 1 ml of ‘cold’ side solution before and after each 1-h test period, whereas $\text{cpm}(H)$ refers to counts per minute of 1 ml of ‘hot’ side solution.

**Morphologic studies**

Distal colon tissue was obtained immediately after heat or detergent injury and after 1 and 2 h in the Ussing chamber. Tissues were fixed in 10% formalin and embedded in paraffin, and sections examined by light microscopy after staining with haematoxylin and eosin.

**Statistics**

All values are expressed as mean ± standard error. The Mann–Whitney two-tailed test was used to assess the significance of differences between means of groups. A $P$ value of less than 0.05 was considered statistically significant.

**Results**

**Thermal injury**

Histologic changes noted immediately after thermal injury were minimal and consisted of focal vacuolation of the surface epithelium. The crypts and lamina propria appeared normal. However, 1 h after injury, there was loss of epithelial cells on the surface of the mucosa, the crypts being generally spared (Fig. 1).

**Table I.** The effect of 25 mM butyrate on tissue electric resistance ($\mu\text{hm}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$) in rat distal colon exposed to heat or detergent injury

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Normal control</th>
<th>Control</th>
<th>Butyrate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$n$ = 6</td>
<td>$n$ = 10</td>
<td>$n$ = 8</td>
</tr>
<tr>
<td>0</td>
<td>103.1 (5.3)</td>
<td>55.2 (6.7)*</td>
<td>94.3 (7.1)†</td>
</tr>
<tr>
<td>60</td>
<td>88.5 (7.8)</td>
<td>30.8 (4.2)*</td>
<td>68.5 (7.0)†</td>
</tr>
<tr>
<td>120</td>
<td>81.9 (8.1)</td>
<td>22.5 (2.5)*</td>
<td>48.3 (6.3)†</td>
</tr>
<tr>
<td>180</td>
<td>69.9 (5.4)</td>
<td>16.9 (2.3)*</td>
<td>35.2 (4.1)†</td>
</tr>
<tr>
<td>240</td>
<td>60.2 (6.5)</td>
<td>12.9 (1.5)*</td>
<td>25.5 (4.8)†</td>
</tr>
</tbody>
</table>

All values are mean (standard error).

* $P < 0.01$ compared with normal control.

† $P < 0.01$ compared with injured control. Injured tissues exposed to butyrate did not differ significantly from uninjured control.

**Table II.** The effect of 25 mM butyrate on serosa-mucosa flux of $^{14}$C PEG-4000 ($\mu$mol/cm$^2$·h$^{-1}$) across rat distal colon exposed to heat or detergent injury

<table>
<thead>
<tr>
<th>Flux period (h)</th>
<th>Normal control</th>
<th>Control</th>
<th>Butyrate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$n$ = 6</td>
<td>$n$ = 8</td>
<td>$n$ = 9</td>
</tr>
<tr>
<td>1</td>
<td>0.02 (0.005)</td>
<td>0.40 (0.06)*</td>
<td>0.26 (0.05)</td>
</tr>
<tr>
<td>2</td>
<td>0.02 (0.005)</td>
<td>0.56 (0.09)*</td>
<td>0.45 (0.07)*</td>
</tr>
<tr>
<td>3</td>
<td>0.09 (0.03)</td>
<td>0.70 (0.10)**</td>
<td>0.46 (0.07)†</td>
</tr>
<tr>
<td>4</td>
<td>0.13 (0.02)</td>
<td>0.93 (0.10)**</td>
<td>0.55 (0.10)†</td>
</tr>
</tbody>
</table>

All values are mean (standard error).

* $P < 0.05$ compared with normal control.

** $P < 0.01$ compared with normal control.

† $P < 0.05$ compared with injured control.

‡‡ $P < 0.01$ compared with injured control.
containing solution had lower electric resistance than control tissue, but resistance was significantly higher than in tissue bathed in butyrate-free solution, at all time periods tested (Table I).

Table II shows passive flux of PEG 4000 across the distal colon in vitro. Flux was very low or negligible across normal colon mucosa and was increased significantly in heat-treated colon at all time periods. Fluxes across heat-treated colon were significantly less in tissues bathed in butyrate-containing solution than in those bathed in butyrate-free solution during the third and fourth time periods (Table II).

Table III compares passive (serosal-mucosal) flux of $^{22}\text{Na}$ across normal and heat-treated colonic mucosa. Heat treatment significantly increased the passive flux of $^{22}\text{Na}$ at all time intervals studied. No significant difference was noted between mucosa in Ringer solution and mucosa exposed to butyrate.

**Detergent injury**

Necrosis and loss of the superficial epithelium was noted at histologic examination immediately after detergent exposure. The bases of crypts and the lamina propria appeared normal (Fig. 2).

As shown in Table I, detergent injury induced a decrease in the electric resistance of the colon, and this further decreased with time. At all time periods the resistance of detergent-injured tissue bathed in butyrate-containing solution was significantly higher than the resistance in butyrate-free solution (Table I).

Passive flux of PEG 4000 across detergent-treated colon was significantly higher than across control tissue (Table II) and increased slightly with time. Flux across tissue bathed in butyrate solution was significantly less than across tissue bathed in butyrate-free solution at all time periods (Table II).

Passive flux of $^{22}\text{Na}$ was significantly increased across detergent-treated colon compared to control tissue (Table III). No significant difference in $^{22}\text{Na}$ flux was observed between tissues bathed in butyrate-free and butyrate-containing solutions (Table III).

**Influence of age of animal on effect of butyrate**

As shown in Table IV and Table V, butyrate increased tissue electric resistance in colon from 2-week-old and 6-week-old rats exposed to either thermal or detergent injury. The effect of butyrate was statistically significant compared

### Table III. The effect of 25 mM butyrate on serosa-mucosa flux of $^{22}\text{Na}$ ($\mu\text{e}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$) across adult rat distal colon exposed to heat or detergent injury

<table>
<thead>
<tr>
<th>Flux period (h)</th>
<th>Normal control ($n=6$)</th>
<th>Heat-injured tissue</th>
<th>Detergent-injured tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control ($n=8$)</td>
<td>Butyrate ($n=10$)</td>
<td>Control ($n=8$)</td>
</tr>
<tr>
<td>1</td>
<td>5.1 (0.9)</td>
<td>7.4 (0.8)</td>
<td>6.7 (0.6)</td>
</tr>
<tr>
<td>2</td>
<td>5.4 (0.4)</td>
<td>12.9 (1.3)*</td>
<td>10.4 (0.9)</td>
</tr>
<tr>
<td>3</td>
<td>8.2 (1.0)</td>
<td>12.3 (1.2)*</td>
<td>13.2 (0.9)*</td>
</tr>
<tr>
<td>4</td>
<td>8.2 (0.8)</td>
<td>16.3 (1.6)*</td>
<td>16.1 (0.6)*</td>
</tr>
</tbody>
</table>

All values are mean (standard error).

* $P < 0.05$ compared with normal control.
with control damaged tissue not bathed in butyrate-containing solution.

Discussion

Superficial injury to the intestine and colon, produced usually by the use of detergents or acid, is a model that has been used to study mucosal repair (12, 15). Restoration of barrier function is the immediate objective of the repair process and occurs through migration of epithelial cells over the denuded basal lamina. This process, which takes place over a period of 1–5 h, has been termed epithelial restitution (11, 12, 15). The present study examined the colonic mucosal restoration of barrier function was significantly enhanced in both types of injury by butyrate.

Superficial injury is characterized by a decreased electric resistance of the tissue, reflecting the fact that the mucosa has become more permeable to ions. The migrating and flattened cells re-form tight junctions to restore the tissue electric resistance towards normal. The present study shows that butyrate influences cell migration at fairly low concentrations. However, it is also possible that studies using intact tissue may require the use of higher butyrate concentrations. The present study did not attempt to examine the effect of different butyrate concentra-

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Normal control (n = 6)</th>
<th>Control (n = 6)</th>
<th>Butyrate (n = 6)</th>
<th>Control (n = 5)</th>
<th>Butyrate (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>58.1 (7.7)</td>
<td>33.4 (3.3)**</td>
<td>39.2 (4.1)††</td>
<td>40.9 (4.0)</td>
<td>56.5 (8.2)</td>
</tr>
<tr>
<td>30</td>
<td>54.9 (3.9)</td>
<td>23.7 (2.5)**</td>
<td>38.3 (3.0)†</td>
<td>29.3 (1.1)*</td>
<td>51.8 (8.6)††</td>
</tr>
<tr>
<td>60</td>
<td>50.2 (5.5)</td>
<td>16.6 (1.6)**</td>
<td>31.3 (3.8)*</td>
<td>21.5 (2.5)**</td>
<td>54.3 (7.1)†</td>
</tr>
<tr>
<td>90</td>
<td>45.7 (5.0)</td>
<td>15.5 (1.3)**</td>
<td>28.8 (1.5)**††</td>
<td>24.3 (3.4)*</td>
<td>48.3 (7.2)††</td>
</tr>
<tr>
<td>120</td>
<td>38.5 (5.3)</td>
<td>12.8 (1.8)**</td>
<td>24.6 (1.8)**††</td>
<td>19.1 (2.2)*</td>
<td>43.1 (4.8)††</td>
</tr>
</tbody>
</table>

All values are mean (standard error).
* P < 0.05 compared with normal control.
** P < 0.01 compared with normal control.
† P < 0.05 compared with injured control.
†† P < 0.01 compared with injured control.

An earlier study failed to show an effect of 10 mM butyrate on epithelial restitution in rat distal colonic mucosa wounded with hydrochloric acid (19). The mode of injury in the former study could have influenced the results. However, the present study had other differences from the previous one, including the presence of butyrate on both mucosal and serosal sides and the use of intact colonic wall (versus preparations stripped of serosa and muscularis in the earlier study). It is also possible that butyrate has a concentration-dependent effect on restitution. In cell monolayers butyrate influences cell migration at fairly low concentrations. However, it is possible that studies using intact tissue may require the use of higher butyrate concentrations. The present study did not attempt to examine the effect of different butyrate concentra-

Table V. Electric resistance (Ω/cm·m²·h⁻¹) of colon from 2-week-old rats after heat or detergent injury

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Normal control (n = 5)</th>
<th>Control (n = 10)</th>
<th>Butyrate (n = 7)</th>
<th>Control (n = 6)</th>
<th>Butyrate (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>84.7 (10.9)</td>
<td>74.6 (2.2)</td>
<td>68.0 (3.7)</td>
<td>55.8 (3.7)*</td>
<td>68.5 (4.6)</td>
</tr>
<tr>
<td>30</td>
<td>63.7 (8.3)</td>
<td>50.5 (4.8)</td>
<td>54.0 (4.0)</td>
<td>39.2 (2.1)*</td>
<td>57.5 (5.9)††</td>
</tr>
<tr>
<td>60</td>
<td>53.4 (7.1)</td>
<td>32.8 (3.4)***</td>
<td>51.0 (4.4)††</td>
<td>29.7 (1.5)**</td>
<td>51.8 (5.6)††</td>
</tr>
<tr>
<td>90</td>
<td>59.2 (6.3)</td>
<td>28.2 (2.9)***</td>
<td>39.3 (4.6)</td>
<td>22.8 (1.6)**</td>
<td>38.3 (4.8)††</td>
</tr>
<tr>
<td>120</td>
<td>66.2 (4.3)</td>
<td>25.4 (1.6)***</td>
<td>34.2 (3.3)*</td>
<td>19.1 (2.1)**</td>
<td>31.1 (4.9)††</td>
</tr>
</tbody>
</table>

All values are mean (standard error).
* P < 0.05 compared with normal control.
** P < 0.01 compared with normal control.
† P < 0.05 compared with injured control.
†† P < 0.01 compared with injured control.

Butyrate Restores Barrier Function
Butyrate has diverse effects on colonic function and on colonic epithelial cells. It is the principal energy substrate for colonocytes (20). However, the effect on epithelial migration is independent of cell metabolism (18). Butyrate also stimulates colonic epithelial cell proliferation (21). The time frame in which restoration of barrier function was noted in the present study makes it unlikely that epithelial proliferation played any part in the butyrate effect. The effect of butyrate on barrier function is most consistent with an effect on epithelial migration. This is supported by the finding, in colonic epithelial cell monolayers, that butyrate stimulates cell migration after wounds have been induced in the monolayer (18). Butyrate is known to have effects on gene transcription in epithelial cells (22). It is likely that the effects of butyrate on epithelial migration and restoration of barrier function are mediated through modulation of gene expression necessary for molecules that are essential to epithelial migration. Various molecules are involved in this process, including extracellular factors such as growth factors and trefoil peptides, molecules that regulate cell–cell or cell–matrix adhesion, molecules that mediate detachment from the substratum, and molecules that regulate cytoskeletal function (11).

Impairment of the mucosal barrier may lead to mucosal inflammation, in turn leading to epithelial damage. The mucosal barrier has been noted in Crohn disease patients and in some of their asymptomatic relatives (5). Altered colonic permeability is also a feature of active ulcerative colitis (3, 4). Butyrate enemas have been shown to have a beneficial effect on colonic mucosa in distal ulcerative colitis (23, 24). It is possible that one of the mechanisms underlying this effect of butyrate is its effect on restoration of the epithelial barrier. Enhancement of epithelial restitution in the colon by butyrate may provide a basis for the use of butyrate enemas in other disease states of the colon characterized by surface epithelial injury.

Acknowledgements

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References


