Butyrate upregulates stromelysin-1 production by intestinal mesenchymal cells

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Pender, Sylvia L. F., Jessica J. Quinn, Ian R. Sanderson, and Thomas T. MacDonald. Butyrate upregulates stromelysin-1 production by intestinal mesenchymal cells. Am J Physiol Gastrointest Liver Physiol 279: G918–G924, 2000.—Nutritional factors and resident bacteria participate in the pathogenesis of intestinal inflammation. However, the ways in which bacteria and complex diets might modulate matrix metalloproteinase (MMP) production are unknown. We hypothesized that butyrate might enhance production of MMPs, thus amplifying their response to signals in inflammatory conditions. Human mesenchymal cells were incubated with butyrate and then stimulated with cytokines. MMPs and inhibitors were studied by Western blotting and quantitative RT-PCR. Acetylation of histones was examined in Triton X acetic acid-urea gels by PAGE. We showed that butyrate selectively enhanced the protein production and mRNA expression of stromelysin-1 in tumor necrosis factor-α- or interleukin-1β-stimulated mesenchymal cells. Butyrate alone did not induce any change in MMP production or mRNA expression. It increased the acetylation of histones in mesenchymal cells. Furthermore, acetylation of histones (induced by trichostatin A) reproduced the effects of butyrate. Although butyrate is a major source of nutrient for the colonic epithelial cells, it modulates intestinal inflammation through the secretion of stromelysin-1 in stimulated stromal cells via the inhibition of histone deacetylase.

MMPs may play an important role in gut inflammation. Their activity is, however, tightly regulated by the tissue inhibitors of metalloproteinase (TIMPs).

There have been few mechanistic studies of MMPs in the gut. We have recently shown that MMPs play an important part in T cell-mediated tissue injury in the gut (23). When resident T cells in human fetal small intestine explants are stimulated with pokeweed mitogen, tissue injury, matrix degradation, and MMP production can be observed in the explants after 72–96 h of culture (20, 22). In addition, activated recombinant stromelysin-1 at nanomolar concentrations causes the same pattern of tissue injury and matrix degradation as seen in the culture explants but in only 24 h. However, activated recombinant interstitial collagenase, gelatinase A, and gelatinase B do not cause any tissue injury. A major source of the MMPs in the fetal gut culture explants are cytokine-activated stromal cells (23). Mucosal injury can be prevented by agents that inhibit T cell activation or cytokine activity or by MMP inhibitors (15, 20, 21, 23).

Although the cause of IBD is unknown, there is increasing evidence from patient and animal models that dietary factors and bacteria are important in its pathogenesis. It has been reported, for example, that IL-2 (26) or IL-10 (14) knockout mice develop severe colonic inflammation. This tissue injury does not occur when animals are kept in a germ-free environment, demonstrating that the resident bacterial flora play an important but as yet undefined role. The way in which bacterial flora modulate the disease process is unknown. However, one of the key interactions between nutrition and the flora is the production of short-chain fatty acids. The concentration of butyrate, in particular, depends on the fermentation of nonabsorbed carbohydrates by bacteria in the intestinal lumen. Butyrate is an inhibitor of proliferation and an inducer of cell differentiation in numerous tumor cell lines (2). It acts through the inhibition of histone deacetylase to alter the transcription of a number of genes (7, 12, 13, 30), and it is established that hyperacetylation is the

MATRIX METALLOPROTEINASES (MMPs) are a group of Ca2+-dependent and Zn2+-containing enzymes produced by various cell types and are capable of degrading all components of the extracellular matrix (ECM) (17, 34). Excess MMP activity causes various kinds of tissue injury in conditions such as rheumatoid arthritis, osteoarthritis, periodontal diseases, tumor progression, and bone resorption (16). Recently, it has been shown that MMPs also play an important role in tissue degradation in inflammatory bowel disease (IBD) (1, 3, 25, 32). In Crohn’s and ulcerative colitis, there are increased amounts of MMP transcripts and protein in the mucosa compared with controls, suggesting that MMPs may play an important role in gut inflammation. Their activity is, however, tightly regulated by the tissue inhibitors of metalloproteinase (TIMPs).

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mechanism by which butyrate causes induction of differentiation and growth arrest of cells (6).

Sodium butyrate is also a major source of nutrient for the colonic epithelial cells (33). Epithelial cells can convert butyrate into energy through β-lipolysis and the Krebs cycle. Butyrate facilitates salt and water absorption and reduces epithelial permeability in the colon, and it is sometimes used as a treatment of ulcerative colitis. A multicenter study in the United States showed that butyrate is efficacious only in early lesions of ulcerative colitis, in which epithelial cell loss results in loss of mucosal barrier function (4). But it is of no benefit in Crohn’s disease, in which butyrate concentrations are increased (31).

We therefore hypothesized that, although the main source of butyrate is from epithelial cells, it might enhance MMP production of stromal cells, leading to gut inflammation. In this study we have demonstrated that butyrate specifically enhanced the production of stromelysin-1 protein and mRNA in cytokine-stimulated mesenchymal cells. Butyrate also enhanced the acetylation of histones extracted from the mesenchymal cells. In addition to this, a specific inhibitor of histone deacetylase also increased stromelysin-1 production by stimulated cells. This is the first study to show that the production of stromelysin-1 is regulated not only by transcriptional factors but also by histone acetylation.

MATERIALS AND METHODS

Second-trimester human fetal small intestine was obtained within 2 h of surgical termination from the Medical Research Council Tissue Bank, the Hammersmith Hospital (London, UK). This study received ethical approval from the Hackney and District Health Authority (London, UK). Isolation and characterization of mucosal mesenchymal cells. Human fetal mesenchymal cells were isolated and characterized according to the methods described previously (23). Only cells that grew to passage 4 and beyond were used, at which stage the numbers of epithelial cells and macrophages were minimal. Each batch of cells was characterized before use. Mesenchymal cells (5 × 10⁵) were seeded into 25-cm² culture flasks and maintained in MEM and 10% FCS overnight. After 24 h, the cell layer was washed twice with PBS and preincubated with sodium butyrate (1.5 mM or 5 mM; Sigma Chemical, Poole, UK) for 24 h. These concentrations were chosen according to our previous study on butyrate effect on epithelial cells (8, 19). The cell layer was washed again with PBS before stimulation with either interleukin (IL)-1β or tumor necrosis factor (TNF)-α (1 ng/ml; Sigma) 3 h after IL-1β or TNF-α stimulation (Fig. 1). Culture supernatants were collected, and total RNA was isolated at 3, 6, 12, and 24 h. MMP mRNA expression was examined by quantitative RT-PCR.

Histone extraction and separation. Histones were extracted from mesenchymal cells according to Cousins and Alberts (5). In brief, cells were plated in 75-cm² flasks and maintained in MEM with 10% FCS until confluence. After 24-h incubation with either butyrate (5 mM), trichostatin A (TSA, 10 μM; Sigma) (35), or media alone, cells from each treatment were removed and the nuclear protein was harvested by centrifuging in MLB buffer (60 mM KCl, 15 mM NaCl, 3 mM MgCl₂, 15 mM PIPES, pH 6.5, 0.1% Nonidet P-40, 0.5 M phenylmethylsulfonyl fluoride, and 1 mM tetra- thionate). The nuclear pellet was resuspended in H₂SO₄ to a final concentration of 0.2 M for 2 h at 4°C. The dissolved histones were precipitated with alcohol at -20°C. The precipitant was then resuspended in water and quantified, and 200 μg of nuclear protein from each sample was suspended in running buffer before being loaded onto a Trition X acetic acid-urea gel for histone separation. The gel was prepared by layering an upper gel [1 M acetic acid, 6.3 M urea, and 4.4% (wt/vol) acrylamide] onto a separating gel [1 M acetic acid, 8 M urea, 0.5% (vol/vol) Triton X-100, 45 mM NH₄Cl, and 16% (wt/vol) acrylamide]. Gels were stained with Coomassie brilliant blue and destained, and the position of the histone 4 was identified with a histone 4 marker (Boehringer Mannheim).

RESULTS

Effects of sodium butyrate on MMP production and mRNA expression by proinflammatory cytokine-stimulated mesenchymal cells. In medium alone, mesenchymal cells secrete low amounts of interstitial collagenase, stromelysin-1, and gelatinase B. However, after stimulation with IL-1β or TNF-α, stromelysin-1 and gelatinase B production were dramatically increased.
and interstitial collagenase production was slightly upregulated, but gelatinase A and TIMP-1 were unchanged. With the addition of increasing concentrations of sodium butyrate for 24 h, stromelysin-1 production by cytokine-stimulated cells was specifically enhanced, whereas the production of interstitial collagenase and gelatinase B was decreased. Gelatinase A and TIMP-1 production remained unchanged (Fig. 2A). When cellular protein levels were measured, we again found that low amounts of stromelysin-1 and interstitial collagenase were detected when the cells were unstimulated but that production was dramatically upregulated after stimulation with IL-1β or TNF-α. When the cells were pretreated with increasing concentrations of butyrate for 24 h, the production of stromelysin-1 cellular protein was enhanced, whereas interstitial collagenase was decreased after being stimulated by cytokines (Fig. 2B). When examined at earlier time points, the production of stromelysin-1 started at 6 h, whereas the production of interstitial collagenase only started at 12 h (Fig. 3). At such time points, the dose-dependent response to sodium butyrate was particularly evident. Butyrate alone had no effect on MMP production (data not shown). Sodium butyrate with or without IL-1β or TNF-α also had no effect on mucosal mesenchymal cell proliferation (data not shown).

The effects of sodium butyrate on MMP mRNA expression correlated with protein production. With the use of competitive RT-PCR, stromelysin-1 mRNA rapidly increased, peaking at 3 and 6 h after stimulation with IL-1β and TNF-α, respectively, after which the expression decreased. With the addition of sodium butyrate, this response was enhanced in a dose-dependent manner (Fig. 4). However, butyrate had a different effect on the interstitial collagenase mRNA expression. When the cells were stimulated with IL-1β, the highest mRNA level was detected at 6 h and remained high at 12 h. When the cells were stimulated with TNF-α, the mRNA expression was still increasing at 12 h. In contrast to stromelysin-1 mRNA expression, interstitial collagenase mRNA expression was downregulated by sodium butyrate (Fig. 4).

Butyrate does not increase the stability of stromelysin-1 mRNA. The increased stromelysin-1 protein production and mRNA expression could be explained either by an increase in the stability of mRNA or by an increase in the transcription of the gene. Transcription was therefore interrupted with actinomycin D 3 h after the mesenchymal cells were stimulated with IL-1β when stromelysin-1 mRNA had reached its peak. Under these circumstances, stromelysin-1 mRNA decayed at an equal rate both in the cells that had received butyrate before stimulation with IL-1β and in the cells

![Fig. 2. Effects of butyrate on MMP protein production in cytokine-stimulated mesenchymal cell culture supernatants (A) and cellular extracts (B). Sodium butyrate specifically enhanced both IL-1β- and TNF-α-stimulated stromelysin-1 production by mesenchymal cells in a dose-dependent manner. However, production of interstitial collagenase and gelatinase B was suppressed by butyrate. Gelatinase A and tissue inhibitor of metalloproteinase (TIMP)-1 production showed no change. Culture supernatants and cellular extracts were collected at 48 h after cytokine stimulation with or without preincubation with sodium butyrate. This Western blot is representative of 4 individual experiments.](http://ajpgi.physiology.org/)

![Fig. 3. Effects of butyrate on MMP production by mesenchymal cells stimulated with IL-1β (A) or TNF-α (B) at different time points. Changes in stromelysin-1 production were not observed with butyrate unless cells were treated with cytokines for at least 6 h. At 6, 12, and 24 h, clear dose- and time-dependent responses were seen. This Western blot is representative of 3 individual experiments.](http://ajpgi.physiology.org/)
that received IL-1β alone (Fig. 5). Thus the stability of stromelysin-1 mRNA was not enhanced by butyrate, and its increase was therefore due to increased transcription.

**Butyrate and TSA induce histone acetylation in small intestine mesenchymal cells and increase stromelysin-1 production.** Butyrate is an inhibitor of histone deacetylase. Although butyrate is found in appreciable quantities only in the intestine, its effect on histone acetylation in intestinal stromal cells has not been examined. Addition of 5 mM sodium butyrate for 24 h to these cells induced acetylation of histones, as seen by the different forms of histone 4 (Fig. 6A). TSA, a specific inhibitor of histone deacetylase, also resulted in an increase in the acetylation of histones in mesenchymal cells (Fig. 6A).

TSA had the same effects on MMP production by cytokine-stimulated mesenchymal cells as described for sodium butyrate. It also specifically enhanced stromelysin-1 production and downregulated interstitial collagenase production after cytokine stimulation for 48 h (Fig. 6B). TSA also had the same effect as butyrate, downregulating gelatinase B production but having no effect on either gelatinase A or TIMP-1 production (data not shown).

**DISCUSSION**

To the best of our knowledge, this study shows for the first time that histone acetylation can regulate the production and mRNA expression of MMPs by cytokine-stimulated gut mesenchymal cells when stimulated with either IL-1β or TNF-α. This study also demonstrates that sodium butyrate, a metabolite derived from bacterial fermentation of unabsorbed carbohydrate, specifically enhances the production of stromelysin-1 in stimulated cells. Butyrate decreases the production of interstitial collagenase and gelatinase B, whereas that of gelatinase A and TIMP-1 shows no change. Butyrate in the absence of proinflammatory agents has no action, but it amplifies their action on stromelysin-1 secretion through histone acetylation, thereby increasing the potential for severe gut damage.

The concentration of butyrate in the intestine ranges from 0 to 16 mM (18). If butyrate is a normal constituent of the intestine that upregulates stromelysin-1,
Butyrate could elevate the production of MMPs and the degradation of ECM and increase gut injury. During the pathogenesis of IBD, bacterial flora and their metabolites may also penetrate the compromised mucosal barrier and further amplify the production of stromelysin-1 by mesenchymal cells. In animal models of IBD, certain genetic knockout mice such as IL-2 and IL-10 (14, 26) develop severe gut inflammation spontaneously. However, when these animals are kept under germ-free conditions, they remain healthy. Recent studies in our laboratory have already shown that there are higher concentrations of stromelysin-1 found in IBD patients than in control groups. We therefore suggest that under certain circumstances, when there is an imbalance of proinflammatory cytokines, a bacterial metabolite, butyrate, whose function in health is to provide energy, can amplify the IL-1β or TNF-α response.

Butyrate has a range of actions in the intestine. It facilitates salt and water absorption in the colon. It is also an energy source for colonocytes and may enhance mucosal restitution and in turn maintain the mucosal barrier. This barrier may become more permeable because of insufficient fuel. Thus butyrate reduces inflammation in patients with diverted colons (11). Numerous in vitro experiments with colon cancer cell lines have shown that butyrate also acts as an inhibitor of growth and an inducer of differentiation markers (6). Moreover, it is also an inducer of apoptosis for non-transformed intestinal epithelial cells (9) as well as for colon cancer cell lines (10). In this study, we also show that butyrate enhances the regulation of stromelysin-1 production in response to cytokines. This leads to degradation of the mucosal ECM and increases mucosal gut injury. This observation may help explain the confusion that exists concerning whether butyrate enemas are beneficial in ulcerative colitis patients. It has only been shown that butyrate has significant benefit in recently diagnosed patients (<6 mo duration) (4). In such patients, epithelial cell loss is evident, and consequently the effect of butyrate on cell turnover becomes more important. Otherwise, several major well-conducted trials have demonstrated that there is no significant effect of short-chain fatty acids, including sodium butyrate, on disease activity or on histology (27, 28). In addition, butyrate has no beneficial effect in patients with Crohn’s disease. In fact, studies on cell lines show that it enhances the secretion of IL-8 (8) and macrophage inflammatory protein-2 (19) in response to IL-1β or lipopolysaccharide. Therefore, the effect of butyrate is a balance between its effects on epithelial cell maturation and its enhancement of proinflammatory signals. In this study, butyrate also induces stromelysin-1 production by mesenchymal cells when stimulated with IL-1β and TNF-α, demonstrating that its amplifying effects are not restricted to epithelial cells.

The present study also showed that the mode of action is through histone acetylation. TSA, a specific inhibitor of histone deacetylase that has no chemical similarity to butyrate, not only causes hyperacetyla-
tion like butyrate but also mimics its growth-inhibiting and differentiation-inducing effects (6). In this study, it has a similar effect to butyrate in regulating MMP production by stimulated gut mesenchymal cells. Its potential for inducing histone acetylation is ~1,000-fold greater than that of butyrate. The amount of TSA required to alter MMP secretion similar to that seen with butyrate was of the order necessary to cause similar changes in histone acetylation.

In conclusion, this study shows that a luminal molecule, butyrate, regulates the molecular mechanisms important in MMP expression. We speculate that this may be one of the pathways by which the luminal environment induced by changes in diet may modify the effects of inflammation in the gastrointestinal tract.

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