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Key role of short-chain fatty acids in epithelial barrier failure during ruminal acidosis

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ABSTRACT

Subacute ruminal acidosis is induced by high concentrations of short-chain fatty acids (SCFA, mainly acetate, propionate, and butyrate) that release protons to decrease the pH of the ruminal digesta. This low pH, in turn, is thought to damage epithelial barrier function. The present study applied a model of simulated ruminal acidosis ex vivo to investigate if SCFA directly contribute to epithelial barrier failure beyond their role as proton donors. Epithelial tissues from the rumen of slaughtered sheep were mounted in Ussing chambers and incubated under 3 different conditions. Two groups were incubated in the absence of SCFA at mucosal pH 6.1 (control) and pH 5.1, respectively, for 7 h. A third group was first incubated in a mucosal solution containing 100 m*M* SCFA at pH 5.1 for 2 h and, thereafter, in a mucosal solution without SCFA at pH 6.1 for the remaining 5 h. Transepithelial conductance (G_t), short-circuit current (I_{sc}), and fluorescein fluxes were determined. After 7 h of incubation, the expression levels of claudin-1, claudin-4, claudin-7, and occludin were measured by quantitative reverse-transcription PCR and Western blot. Furthermore, the local distribution of these tight junction (TJ) proteins was examined by confocal laser scanning microscopy. A 7-h incubation at pH 5.1 in the absence of SCFA did not influence either G_t or fluorescein flux rates of ruminal tissues ex vivo compared with the control. In contrast, incubation at pH 5.1 with SCFA for only 2 h induced increases in G_t and fluorescein flux rates that continued even after tissues were returned back to pH 6.1. Expression analysis showed that pH 5.1 without SCFA for 7 h induced no changes in mRNA expression of claudin-1, claudin-4, claudin-7, and occludin and a selective decrease in protein expression of only claudin-4 compared with the control. However, a 2-h incubation at pH 5.1 in the presence of SCFA decreased the mRNA-expression of claudin-7, as well as the protein expression of claudin-4, claudin-7, and occludin. The decreased expression of these TJ proteins in the group incubated with SCFA was also evident in immunohistochemistry. Immunohistochemistry additionally evidenced a considerable retraction of all tested TJ proteins out of the TJ in that group. We conclude that a low mucosal low mucosal pH of 5.1 is tolerated well by ruminal epithelia for several hours. However, a low pH in combination with SCFA induces damage to the TJ and disturbs barrier function, which is not immediately reversible upon the removal of the acidotic insult.
Key words: ruminal epithelium, subacute ruminal acidosis, tight junction, short-chain fatty acid

INTRODUCTION

Subacute ruminal acidosis is triggered by an excessive intake of highly fermentable feedstuffs as commonly fed to high-yielding dairy cows (Kleen et al., 2003). In the course of this disease, short-chain fatty acids (**SCFA**) accumulate in the rumen due to rapid microbial breakdown of carbohydrates. On the one hand, increased production of acetate (45–70%), propionate (15–40%), and butyrate (5–20%; Bergman, 1990; Aschenbach et al., 2011) is essential to meet the increased energy and glucose demand during lactation (Bugaut, 1987; Aschenbach et al., 2010). On the other hand, an increased ruminal concentration of SCFA leads to a decrease in ruminal pH to values below 5.5. The latter can cause an impairment of the epithelial barrier (Penner et al., 2009; Aschenbach et al., 2011). Histological changes such as parakeratosis indicate a disturbed organization of the epithelium. The epithelial barrier becomes permeable for bacteria and endotoxin (Szemeredy and Raul, 1976), which after translocation can cause liver abscesses and laminitis, followed by a decrease in milk yield, and in severe cases, the animal has to be culled (Nocek, 1997; Tadepalli et al., 2009).

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Transmembrane tight junction (**TJ**) proteins are crucial structures to prevent the translocation of noxious substrates across the epithelial layer. These proteins form circumferential cell-cell contacts that limit the permeability along the paracellular pathway in epithelia and endothelia (Balda and Matter, 2003). Tight junction proteins are subdivided in 2 families; one composed of occludin, tricellulin, and Marvel D3 (Raleigh et al., 2010), whereas the other includes the currently 27 known claudins (Morita et al., 1999; Mineta et al., 2011). Claudins regulate epithelial barrier function in different tissues mostly as sealing (i.e., tightening, components); however, some of them also form pores for charge and size-selective paracellular diffusion. Numerous studies have demonstrated the relevance of deficient TJ protein expression and distribution related to various epithelial diseases (Markov et al., 2015). For example, impaired barrier function and increased loss of solutes and water into the intestinal lumen has been demonstrated in inflammatory bowel disease, which was based on changes in epithelial TJ structure and a reduced tight junctional complexity (Heller et al., 2005; Zeissig et al., 2007). In the stratified squamous epithelium of the rumen, claudins form a barrier against the passive paracellular entry of substances from the ruminal content. So far, claudin-1, claudin-4, claudin-7, and occludin have been identified as relevant TJ components in ruminal epithelia (Stumpff et al., 2011). Whereas claudin-1 and claudin-4 are clearly barrier-forming claudins (Van Itallie et al., 2001; Furuse et al., 2002), the role of claudin-7 is not as well defined. Nonetheless, changes in claudin-7 expression are involved in epithelia dysfunction. For example, the occurrence of typical skin lesions in psoriasis, involving parakeratosis, is linked to a low expression level of claudin-7 (Kirschner et al., 2009) and intestine-specific claudin-7 knockout mice showed increased intestinal permeability and inflammation of mucosal structures (Tanaka et al., 2015).

The relevance of epithelial TJ disruption in the rumen was previously demonstrated in goats (Liu et al., 2013). Goats fed a high-grain diet in vivo showed a decrease in ruminal pH from 6.1 to 5.3 in combination with alterations in ruminal epithelial structure. The altered ruminal epithelial structure, in turn, could be related to a downregulation of claudin-4 and occludin mRNA and protein expression as well as to a redistribution of claudin-1, claudin-4, and occludin (Liu et al., 2013). We had demonstrated previously that similar barrier failure can be induced in isolated ruminal epithelia ex vivo by ~1 h acidification of the mucosal incubation solution to pH 5.1 in the presence of 39 m*M* SCFA (Aschenbach and Gäbel, 2000). In a subsequent trial, however, a similar acidification to pH 5.2 for 1.5 h ex vivo led to no changes in ruminal epithelial permeability when SCFA were absent (Penner et al., 2010). Only when the acidic solution on the mucosal side was replaced by a solution with physiological luminal pH, epithelia showed some minor degree of barrier failure during the recovery period (Penner et al., 2010). From these experimental findings, the hypothesis was generated (1) that the presence of SCFA crucially determines the degree of epithelial damage during an acidotic insult and (2) that the epithelial damage develops to its full extent after the acidotic insult when the pH is already back at physiological levels. To proof this hypothesis, the present study was designed to monitor the early events of barrier failure during a simulated ruminal acidosis ex vivo and to evaluate the changes in the expression and localization of TJ proteins underlying these early events. A special focus was to determine whether the presence of SCFA can aggravate the impairment in barrier function elicited by low pH.

MATERIALS AND METHODS

Animals and Tissue Preparation

Sheep of both sexes were fed a hay-only diet for at least 2 wk before slaughtering. The animals had ad libitum access to water and lick stones. The sheep were stunned with a captive bolt gun and subsequently killed by exsanguination with following removal of the reticulo-rumen from the abdominal cavity. A 300-cm^2 piece of rumen wall was taken from the ventral sac and rinsed in standard buffered solution (10 m*M* NaCl, 24 m*M* $NaHCO_3$, 0.6 m*M* NaH_2PO_4, 2.4 m*M* Na_2HPO_4, 5.5 m*M* KCl, 10 m*M* 2-(*N*-morpholino)ethanesulfonic acid, 1 m*M* L-glutamine, 10 m*M* glucose, 1 m*M* $CaCl_2$, 1.25 m*M* $MgCl_2$, 100 m*M* Na-gluconate; 37°C, pH 7.4). Afterward the epithelium was stripped from the muscle layer and cut into 5 stripes. Tissue stripes were transported in standard buffered solution gassed with carbogen (95% O_2/5% CO_2) at a temperature of 37°C.

Electrophysiological Measurement in Ussing Chambers

Ruminal tissue stripes were cut into squares of approximately 3 × 3 cm size and each mounted between the 2 halves of a conventional Ussing chamber (Aschenbach and Gäbel, 2000). Both serosal und mucosal surfaces of the rumen were equilibrated in 16 mL of a standard buffered solution (for the composition, see the previous section) at pH 7.4 for 30 min. Afterward, the tissues were divided into 3 treatment groups with different mucosal incubation solutions. A first group was incubated at a mucosal pH 6.1 (group pH 6.1; control)