



A single mild episode of subacute ruminal acidosis does not affect ruminal barrier function in the short term

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ABSTRACT

Twenty-four German Merino sheep (72.3 ± 10.1 kg of body weight) were fed an all-hay diet and assigned to either the subacute ruminal acidosis (SARA) treatment ($n = 17$) or sham treatment ($n = 7$). The SARA sheep were orally dosed with a 2.2 M glucose solution to supply 5 g of glucose/kg of body weight, whereas sham sheep received an equal volume of water. Ruminal pH was measured for 48 h before and 3 h after the oral dose. Sheep were then killed and ruminal epithelia from the ventral sac were mounted in Ussing chambers. The serosal-to-mucosal flux rate of partially ³H-labeled mannitol ($J_{\text{mannitol-SM}}$), an indicator of barrier function, was measured while epithelia were exposed to 3 sequential in vitro measurement periods lasting 1 h each. The measurement periods consisted of baseline, challenge, and recovery periods and were interspersed by 30-min periods for treatment equilibration. Baseline conditions were pH 6.1 (mucosal solution) and pH 7.4 (serosal solution) with a bilateral osmolarity of 293 mOsm/L. During the challenge period, the mucosal side of the epithelia was exposed to either an acidotic challenge (pH 5.2, osmolarity 293 mOsm/L) or an osmotic challenge (pH 6.1, osmolarity 450 mOsm/L); a third group served as control (pH 6.1, osmolarity 293 mOsm/L). The mucosal buffer solution was replaced for the recovery period. In vivo, sheep on the SARA treatment had lower mean (5.77 vs. 6.67) and nadir (5.48 vs. 6.47) ruminal pH for the 3 h following the oral drench compared with sham sheep, indicating the successful induction of SARA with the oral glucose dose. Despite the marked reduction in pH in vivo, induction of SARA had no detectable effects on the baseline measurements of $J_{\text{mannitol-SM}}$, tissue conductance (G_t), and short-circuit current (I_{sc}) in vitro. However, reducing mucosal pH

to 5.2 in vitro had negative effects on epithelial barrier function in the recovery period, including increased $J_{\text{mannitol-SM}}$, increased G_t , and decreased I_{sc} . The osmotic challenge increased $J_{\text{mannitol-SM}}$ and G_t and decreased I_{sc} during the challenge period, which was reversible in the recovery period except for slight reduction in I_{sc} . Interactions between the in vitro treatment and measurement period were detected for $J_{\text{mannitol-SM}}$, G_t , and I_{sc} . These data indicate that a mild episode of SARA (nadir pH, 5.48; duration ruminal pH <5.8, 111 min relative to the 180-min measurement period) does not affect ruminal epithelial barrier function immediately after the episode but that a rapid and more severe acidification (pH 5.2) in vitro increases epithelial permeability following the insult.

Key words: barrier function, ruminal acidosis, ruminal epithelium, Ussing chamber

INTRODUCTION

The accumulation and dissociation of short-chain fatty acids (SCFA) in ruminal fluid decreases pH and can lead to the onset of ruminal acidosis (Owens et al., 1998; Plaizier et al., 2008). Because of the current energy-intensive feeding regimens, ruminal acidosis is a persisting disorder in dairy and beef cattle. Ruminal acidosis has negative consequences on feed efficiency through decreased fiber digestibility and impaired production efficiency (Stone, 2004). In addition, ruminal acidosis has been linked to both morphological and histological alterations in ruminal papillae (Steele et al., 2009). The prominent histological alterations during acute and repeated episodes of ruminal acidosis strongly suggest an impaired barrier function (Steele et al., 2009) that may provide the explanation for the translocation of toxins and bacteria during the disorder (Plaizier et al., 2008).

Past studies examining the effect of pH on ruminal epithelial function have consistently demonstrated that epithelial exposure to pH values commonly used for the diagnosis of acute ruminal acidosis (pH ≤ 5.1) results in the rapid reduction of epithelial barrier function

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(Gaebel et al., 1987; Gaebel et al., 1989; Aschenbach and Gäbel, 2000). In studies with a chronic exposure to low ruminal pH, both morphological and histological alterations in ruminal papillae were evident, suggesting reduced barrier function (Steele et al., 2009). Although there is fundamental knowledge of the consequences of acute ruminal acidosis on ruminal epithelial function (Ahrens, 1967; Gaebel et al., 1987; Gaebel and Martens, 1988), only a limited number of studies have examined the functional consequences of a transient and mild acidotic challenge (i.e., SARA) on the ruminal epithelia. However, it is SARA that constitutes the most prevalent form of ruminal acidosis in current dairy production systems (Krause and Oetzel, 2006).

Past studies examining ruminal epithelia function after exposure to low pH have largely focused on absorptive functions. For example, Gaebel and Martens (1988) showed that under washed reticulorumen conditions, an exposure of epithelia to a luminal pH of 5.4 only transiently decreased Na^+ net absorption and transmural potential difference. In an *in vitro* study, Gaebel et al. (1989) demonstrated that mucosal exposure to buffer with a pH value of 6.0 had no effect on short-circuit current (I_{sc}) or tissue conductance (G_t) but further reduction to pH 5.5 decreased I_{sc} and increased G_t , suggesting reduced ion transport and increased epithelial permeability, respectively. With respect to barrier function, Emmanuel et al. (2007) demonstrated that a mucosal pH of 5.5 *in vitro* had no effect on mannitol or lipopolysaccharide translocation across the ruminal epithelium, whereas Aschenbach and Gäbel (2000) showed that a mucosal exposure to pH 5.4 increased the mucosal-to-serosal flux of histamine across ruminal epithelia *in vitro*.

Because ruminal acidosis entails more than simply reducing ruminal pH [e.g., increased osmolarity (Carter and Grovum, 1990; Owens et al., 1998) and increased SCFA and toxin concentrations (Plaizier et al., 2008)], it should be acknowledged that any one or the combination of these factors may affect epithelial barrier function. Past studies have investigated the specific effect of low pH (typical of acute ruminal acidosis), hyperosmolarity (Gaebel et al., 1987; Gaebel and Martens, 1988; Schweigel et al., 2005; Lodemann and Martens, 2006), or an exposure to toxins (Aschenbach and Gäbel, 2000; Emmanuel et al., 2007) *in vitro*. Because SARA rather than acute ruminal acidosis is common in dairy cattle (Krause and Oetzel, 2006), the objective of this study was to elucidate whether exposure of the ruminal epithelium to a short episode of SARA *in vivo* has persistent effects on the barrier function. We further aimed to determine whether such persistent effects could include altered responses to subsequent episodes of mucosal acidity and hyperosmolarity *in*

vitro. We hypothesized that inducing SARA *in vivo* would compromise ruminal epithelial barrier function, with subsequent *in vitro* challenges leading to a further reduction in barrier function.

MATERIALS AND METHODS

This is one paper in a series arising from a single experiment that aimed to evaluate the susceptibility of individual animals to ruminal acidosis. As such, detailed experimental procedures have been described previously (Penner et al., 2009b). This study was conducted between April and August 2008 at the Universität Leipzig (Leipzig, Germany). All procedures were preapproved by the Regierungspräsidium Leipzig (TVV 06/08) and the Faculty Animal Policy and Welfare Committee at the University of Alberta (Edmonton, Alberta, Canada) and were in accordance with the guidelines of the Canadian Council of Animal Care (Ottawa, Ontario, Canada).

Animals and Experimental Design

Twenty-four German Merino sheep (72.3 ± 2.6 kg of BW; mean \pm SD) were used as a model for ruminants. Sheep were sourced from 2 locations and were fed an all-hay diet *ad libitum* for at least 21 d before the start of the experiment. On a DM basis, the hay contained 13.1% CP and 8.1 MJ/kg of ME. Sheep had free access to water and a salt and mineral block.

Prior to the experiment, sheep were transferred to a pen bedded with wood shavings. Hay, water, and mineral block were withdrawn at 0600 h and sheep were randomly exposed to either the control treatment (referred to as sham; $n = 7$) or the SARA challenge treatment (referred to as SARA; $n = 17$). Sheep were weighed and SARA was induced using a ruminal infusion of a 2.2 M glucose solution to supply 5 g of glucose/kg of BW. The infusion was administered using an orogastric tube (12 mm o.d., 150 cm long; Heiland Vet GmbH, Hamburg, Germany). Sheep receiving the sham treatment were exposed to the same procedure but received an equivalent volume of water instead of glucose solution.

Continuous Ruminal pH Measurement *in Vivo*

The protocol for the measurement of ruminal pH in these sheep has previously been reported (Penner et al., 2009b). Briefly, an orally dosable small ruminant ruminal pH measurement system (Penner et al., 2009a; Dascor, Escondido, CA) was used to measure ruminal pH starting 48 h before the oral drench extending for 3 h following the oral drench (Penner et al., 2009b).

Standardization of the pH sensors was performed before the insertion into the rumen and after removal in pH buffers 7.0 and 4.0 (Sensorex, Garden Grove, CA) at 39°C. Regression equations from each standardization (starting and ending standardizations) were used to convert millivolt values to pH units accounting for sensor drift. The sampling rate was 1 reading/2 min, and data for the pre- and postinfusion values were summarized separately.

Ussing Chamber Measurements

Sheep were killed 3 h after the oral drench (i.e., 0900 h) and ruminal epithelia from the ventral sac were collected and prepared for use in Ussing chamber experiments as described previously (Penner et al., 2009b). The serosal buffer solution (pH 7.4) contained (mmol/L) 15.6 NaCl, 5.5 KCl, 1.0 CaCl₂, 1.3 MgCl₂, 0.6 NaH₂PO₄, 2.4 Na₂HPO₄, 1.0 L-glutamine, 10.0 HEPES free acid, 24.0 NaHCO₃, 5.0 Na-D/L-lactate, 10.0 Na-acetate, 10.0 Na-propionate, 10.0 butyric acid, 10 NaOH, and 120.0 mannitol. The mucosal buffer solution was identical to the serosal solution except that 20 mmol/L of mannitol was replaced by gluconic acid to achieve pH 6.1. Different pH values for the mucosal and serosal buffer solutions were used to mimic physiological values. Buffers were continuously gassed with 95% O₂/5% CO₂ (Air Liquide Deutschland GmbH, Düsseldorf, Germany). All epithelia were incubated under short-circuit conditions as described below. Radioactivity (³H]-mannitol, 100 kBq/15 mL; Perkin Elmer, Boston, MA) was added to the serosal solution 20 min after mounting ruminal epithelia in Ussing chambers and a 40-min isotope equilibration period was provided.

Ussing Chamber Experimental Design. After isotope equilibration, epithelia were exposed to a sequential in vitro protocol consisting of three 1-h flux periods (baseline, challenge, and recovery) to measure the serosal-to-mucosal mannitol flux rate ($J_{\text{mannitol-SM}}$) and G_t as indicators of epithelial barrier function and I_{sc} as a measure for active ion transfer across the epithelium (Lodemann and Martens, 2006). The 3 flux periods allowed us to evaluate the effect of the in vivo SARA event (baseline measurement period) and subsequent exposure to mucosal hyperosmolarity or acidity (challenge period) and persistent effects of the in vitro challenge (recovery period). Each 1-h flux period was interspersed by a 30-min interval that was used to apply the in vitro treatments and allow for treatment equilibration. As such, the total in vitro experiment (treatment administration, equilibration, and flux periods) lasted 5 h.

During the in vitro challenge period, the mucosal side of the epithelia was exposed to either an acidotic

challenge (**ACID**) by adding 150 μ L of 1.5 mol/L of gluconic acid or an osmotic challenge (**OSM**) by adding 0.405 g of mannitol; a third group served as control (**CON**). The final pH and osmolarity values for the mucosal buffers were, respectively, 5.2 and 297 mOsm/L for ACID, 6.1 and 450 mOsm/L for OSM, and 6.1 and 293 mOsm/L for CON. To evaluate the recovery following the challenge, the mucosal buffer solution was replaced at the beginning of the 30-min period before the recovery period.

Measurement of Epithelial Barrier Function.

To measure the $J_{\text{mannitol-SM}}$, 800- μ L samples of mucosal buffer solution ("cold side") were collected at the start and end of each flux period and an equivalent volume of buffer was replaced. Samples (100 μ L) of the serosal buffer solution ("hot side") were collected at the start of the baseline and at the end of the recovery periods. The [³H]-mannitol radioactivity was measured in samples from the mucosal and serosal incubation buffers using a scintillation counter (Wallac 1409 LSC; Berthold, Bad Wilbach, Germany). Subsequently, the $J_{\text{mannitol-SM}}$ was determined according to Gäbel et al. (1991).

Electrophysiological parameters were also used as indicators of epithelial barrier function. The measurements of transepithelial potential difference, I_{sc} , and G_t have previously been described (Aschenbach et al., 2000b). Briefly, all epithelia were incubated under short-circuit conditions and the transepithelial potential difference was measured using Argenthal reference electrodes (Mettler Toledo, Urdorf, Switzerland) that were connected to each half (serosal and mucosal) of a Ussing chamber using agar bridges (3% agar in 3 mol/L of KCl). Current was applied using a voltage clamp device (Ing.-Büro für Mess- und Datentechnik, Aachen, Germany) to clamp the transepithelial potential difference to 0 mV. The required current is equivalent but has an opposite direction to the I_{sc} . Tissue conductance was determined according to Ohm's law by measuring the impulse-induced change in the transepithelial potential difference following the application of short bipolar current impulses.

Statistical Analysis

In vivo ruminal fermentation data and baseline values for $J_{\text{mannitol-SM}}$, G_t , and I_{sc} were analyzed as a randomized complete block design using the MIXED procedure of SAS (version 9.1.3, SAS Institute Inc., Cary, NC). The model included the fixed effects of in vivo treatment (sham vs. SARA) and block (source of sheep). Data were presented as least squares means with weighted standard error of the mean. Differences between treatments were considered significant when $P < 0.05$.

Table 1. Body weight and ruminal pH characteristics for sheep receiving an oral drench of glucose to induce SARA (5 mg of glucose/kg of BW) or a volume-equivalent oral drench of water (sham)

Variable	In vivo treatment		Weighted SEM	P-value
	Sham (n = 7)	SARA (n = 17)		
BW, kg	74.9	71.3	2.6	0.399
Predrench ruminal pH over 48 h				
Nadir	5.98	6.11	0.1	0.435
Mean	6.47	6.54	0.07	0.533
Maximum	6.93	7.02	0.07	0.438
Postdrench ruminal pH over 3 h				
Nadir	6.47	5.48	0.05	<0.001
Mean	6.67	5.77	0.06	<0.001
Maximum	6.94	6.524	0.08	0.003
Duration pH <5.8, min	0.4	111.3	13	<0.001
Area pH <5.8, pH × min	0	26.7	5.7	0.007

For the Ussing chamber studies, data were analyzed using the MIXED procedure of SAS (version 9.1.3, SAS Institute Inc.) as a split-plot design. The model included the fixed effects of block, in vivo treatment, in vitro treatment, in vitro measurement period, and the 2 and 3-way interactions. Sheep nested within block × in vivo treatment was included as a random effect. In vitro measurement period was included as a repeated measure and the covariance error structure that yielded the lowest Akaike's and Bayesian information criterion values was used. Significance was declared when $P < 0.05$ with treatment means being separated using the Bonferroni mean separation test.

There were no detectable 3-way interactions, but numerous in vitro treatment × period interactions were present. Therefore, the effects of in vitro treatments are presented without separating the effects between the sham and SARA treatments.

RESULTS

In Vivo Ruminal pH

Ruminal pH characteristics before the oral drench did not differ between sham and SARA sheep (Table 1). However, administering the oral drench markedly reduced the nadir, mean, and maximum ruminal pH values in SARA sheep compared with CON sheep. In

fact, SARA sheep had a nadir ruminal pH almost 1 pH unit lower than CON sheep. Nadir pH for SARA sheep was reached within 128 ± 11.4 min (mean ± SEM) after the glucose drench and SARA sheep spent more than 110 min with a ruminal pH below pH 5.8 following the oral drench.

Epithelial Barrier Function

Although ruminal pH was lower for SARA sheep relative to CON sheep, the in vivo treatment did not affect barrier function of ruminal epithelia measured in Ussing chambers (Table 2). The $J_{\text{mannitol-SM}}$ and G_t averaged $0.64 \mu\text{mol}/(\text{cm}^2 \times \text{h})$ and $1.98 \text{ mS}/\text{cm}^2$, respectively, across in vivo treatments. Short-circuit current as a measure of active ion transfer was also not different between in vivo treatments and had an overall mean of $0.37 \mu\text{Eq}/(\text{cm}^2 \times \text{h})$.

The $J_{\text{mannitol-SM}}$ was affected by the in vitro treatment ($P < 0.001$) and in vitro measurement period ($P < 0.001$; data not shown) with an interaction between the in vitro treatment and in vitro measurement period for the $J_{\text{mannitol-SM}}$ (Figure 1). During the baseline period, there were no differences among treatments for $J_{\text{mannitol-SM}}$. However, during the challenge period, OSM epithelia had greater $J_{\text{mannitol-SM}}$ than CON epithelia, whereas the $J_{\text{mannitol-SM}}$ for ACID epithelia did not differ when compared with CON or OSM. After replacing

Table 2. Baseline measurements of serosal-to-mucosal mannitol flux rates ($J_{\text{mannitol-SM}}$), tissue conductance (G_t), and short-circuit current (I_{sc}) in Ussing chambers from sheep receiving an oral drench of glucose to induce SARA (5 mg of glucose/kg of BW) or a volume-equivalent oral drench of water (sham)

Variable	In vivo treatment		Weighted SEM	P-value
	Sham (n = 7)	SARA (n = 17)		
$J_{\text{mannitol-SM}}$, $\mu\text{mol}/(\text{cm}^2 \times \text{h})$	0.57	0.71	0.06	0.210
G_t , mS/cm^2	1.87	2.08	0.14	0.492
I_{sc} , $\mu\text{Eq}/(\text{cm}^2 \times \text{h})$	0.36	0.37	0.05	0.770

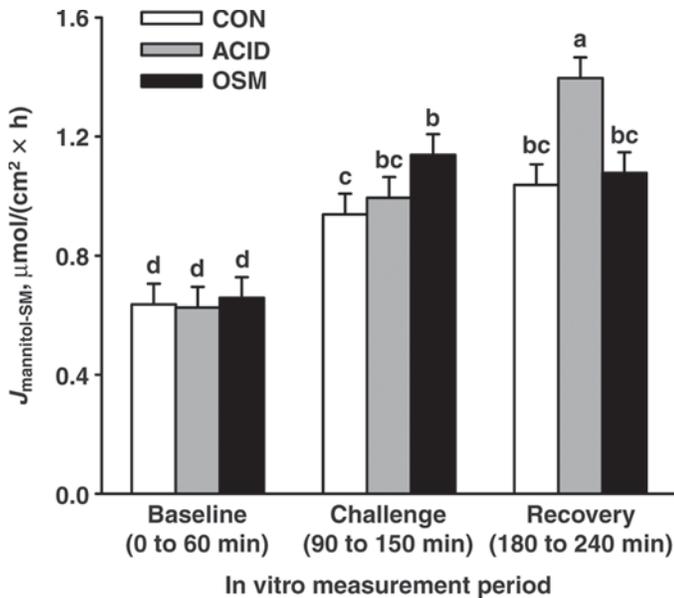


Figure 1. In vitro measurement period \times in vitro treatment interaction for the serosal-to-mucosal mannitol flux rate ($J_{\text{mannitol-SM}}$) across the isolated ovine ruminal epithelia ($n = 24$). Epithelia were subjected to three 1-h flux measurement periods consisting of baseline, challenge, and recovery periods that were interspersed by 30-min periods for treatment equilibration. Baseline conditions were pH 6.1 (mucosal solution) and pH 7.4 (serosal solution) with a bilateral osmolarity of 293 mOsm/L. During the challenge period, the mucosal side of the epithelia was exposed to either an acidotic challenge (ACID; pH 5.2, osmolarity 293 mOsm/L) or an osmotic challenge (OSM; pH 6.1, osmolarity 450 mOsm/L); a third group served as control (CON; pH 6.1, osmolarity 293 mOsm/L). The mucosal buffer solution was replaced for the recovery period. Significant effects were detected for the in vitro treatment ($P < 0.001$), in vitro measurement period ($P < 0.001$), and in vitro treatment \times in vitro measurement period ($P < 0.001$). Means with different letters (a, b, c, and d) differ significantly ($P < 0.05$).

the mucosal buffer solution (i.e., recovery period), the $J_{\text{mannitol-SM}}$ was not different between CON and OSM but was greatest for ACID.

Tissue conductance tended to be affected by in vitro treatment ($P = 0.064$) and changed with in vitro measurement period ($P < 0.001$; data not shown). An interaction between the in vitro treatment and measurement period was detected ($P < 0.001$; Figure 2). Epithelial G_t did not differ among treatments during the baseline period but increased for OSM relative to CON and ACID during the challenge period. The increase in G_t observed for OSM epithelia during the challenge period was reversible as no differences in G_t were found between CON and OSM epithelia in the recovery period. However, ACID epithelia had greater G_t than CON and OSM during the recovery period.

Epithelial I_{sc} was also affected by in vitro treatment, in vitro measurement period, and an in vitro treatment \times in vitro measurement period interaction ($P < 0.001$).

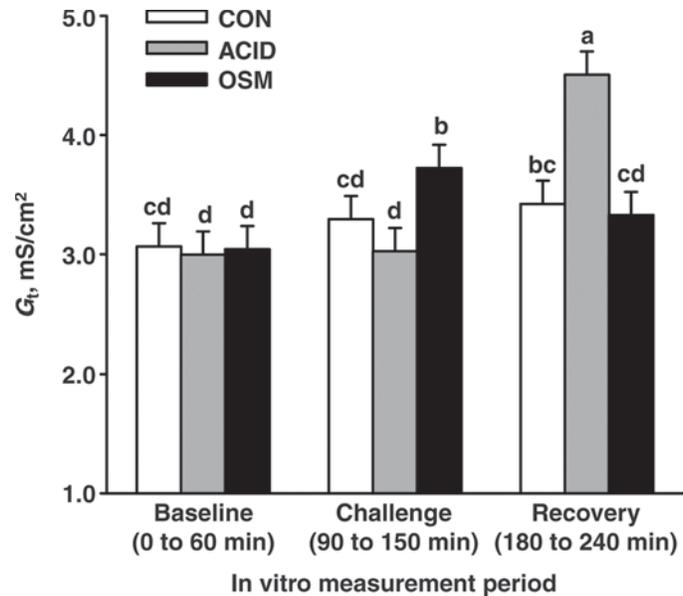


Figure 2. In vitro measurement period \times in vitro treatment interaction for tissue conductance (G_t) of ovine ruminal epithelia ($n = 24$). Epithelia were subjected to three 1-h flux measurement periods consisting of baseline, challenge, and recovery periods that were interspersed by 30-min periods for treatment equilibration. Baseline conditions were pH 6.1 (mucosal solution) and pH 7.4 (serosal solution) with a bilateral osmolarity of 293 mOsm/L. During the challenge period, the mucosal side of the epithelia was exposed to either an acidotic challenge (ACID; pH 5.2, osmolarity 293 mOsm/L) or an osmotic challenge (OSM; pH 6.1, osmolarity 450 mOsm/L); a third group served as control (CON; pH 6.1, osmolarity 293 mOsm/L). The mucosal buffer solution was replaced for the recovery period. Tendencies or significant effects were detected for the in vitro treatment ($P = 0.064$), in vitro measurement period ($P < 0.001$), and in vitro treatment \times in vitro measurement period ($P < 0.001$). Means with different letters (a, b, c, and d) differ significantly ($P < 0.05$).

The in vitro treatment \times in vitro measurement period interaction is reported in Figure 3. There were no differences among treatments during the baseline measurement period; however, administration of gluconic acid or mannitol reduced I_{sc} for OSM epithelia and even more for ACID epithelia. Following replacement of the mucosal buffer solution, I_{sc} remained lowest for ACID, intermediate for OSM, and greatest for CON.

DISCUSSION

Ruminal acidosis occurs when the rate of fermentation acid production exceeds the rate of acid removal from the rumen (Allen, 1997). The severity of ruminal acidosis varies on a continuum from subacute to acute; however, the pH thresholds used to define such events, especially SARA, differ among researchers. Commonly used pH thresholds for SARA range between 5.5 and 5.8 (Kleen et al., 2003; Krause and Oetzel, 2006; Penner et al., 2007) and in some instances include a duration be-

low the threshold (e.g., pH <5.6 for >3 h; Gozho et al., 2005), area below the pH threshold, or area below the pH threshold after being normalized for DMI (Penner et al., 2009c).

Based on a pH threshold of 5.8 or 5.5 (Penner et al., 2007), we successfully induced a mild episode of SARA by administering a glucose solution into the rumen, as indicated by a mean pH of 5.77 and a nadir pH of 5.48. Furthermore, although the total challenge duration was only 180 min, the mean duration for the time spent below pH 5.8 was 111 min, indicating a sustained reduction in ruminal pH. Krehbiel et al. (1995) used a similar challenge model and induced acidosis of increasing severity by applying 6, 12, or 18 g of glucose/kg of BW. Because we aimed at a mild episode of SARA, our glucose dose (5 g/kg of BW) was slightly below the lowest dose used by Krehbiel et al. (1995). Other approaches to induce ruminal acidosis include altering the forage-to-concentrate ratio (Penner et al., 2009c), rapid grain adaptation (Steele et al., 2009), or short-term feed restriction followed by feeding a highly fermentable grain source (Dohme et al., 2008). These alternative approaches to induce SARA differ from the present approach in that they include intermediate to long-term changes in ruminal epithelial function through nutrient deprivation (Gaebel et al., 1987; Gäbel et al., 1993) or nutrient excess (Gaebel et al., 1987; Penner et al., 2009c).

The negative effects of low pH on fiber digestion have been well characterized (Calsamiglia et al., 2002), whereas there is currently a paucity of data examining the effect of SARA on ruminal epithelial barrier function. Acute ruminal acidosis (pH \leq 5.1) is known to decrease epithelial barrier function, which may increase the translocation of pathogenic bacteria such as *Fusobacterium necrophorum* across the ruminal epithelium (Owens et al., 1998; Tadepalli et al., 2009). There has been a renewed interest in measuring barrier function of the ruminal epithelium in relation to potential translocation of toxins and antigens (Aschenbach et al., 2000a; Aschenbach and Gäbel, 2000; Emmanuel et al., 2007) and the activation of the acute phase protein response (Gozho et al., 2005; Plaizier et al., 2008).

When planning the experiments, we acknowledged that the reduction in pH is not the only challenge during SARA. Simulating the complex changes in the rumen by instilling glucose was considered to be an appropriately standardized model to reproduce these complex changes. It was hypothesized that the glucose-induced SARA would reduce epithelial barrier function for a sustained period of time, implying that impaired barrier function could still be detected in vitro after killing the animal. However, we did not observe an increase in either the passive ion leak (i.e., G_t) or in the pas-

sive permeability to the hydrophilic molecule mannitol ($J_{\text{mannitol-SM}}$; commonly used as a marker for paracellular permeability; Schweigel et al., 2005). Strictly speaking, we cannot rule out that a failure of barrier function occurred at the time of SARA in vivo. However, this is not very likely because the in vitro ACID challenge (pH 5.2) demonstrated that barrier failure becomes prominent early after the challenge rather than during the challenge (see below). Our results are congruent with earlier studies using the washed reticulorumen (Gaebel and Martens, 1988), which simulated SARA (pH 5.4; 80 mM SCFA) for a period of 60 min. In that study, they showed that transport activity was only transiently reduced after buffer pH increased from 5.4 to 6.0.

The absence of barrier dysfunction with the chosen SARA induction protocol likely suggests that additional insults or an increased severity of the insult are required to induce sustained epithelial barrier dysfunction. The literature supports the view that, apart from pH, major additional insults are parakeratosis and epithelial inflammation resulting from prolonged exposure to high concentrations of SCFA in the rumen and insufficient physically effective fiber (Nocek, 1997; Kleen et al., 2003; Steele et al., 2009). Additionally, it may be possible that repeated episodes of ruminal acidosis are required to induce changes in epithelial function. In fact, past studies have demonstrated that the severity of the acidosis challenge increases with consecutive challenges (Dohme et al., 2008). Because we worked with sheep not adapted to a high-grain diet, these insults were not part of the experimental model.

In vitro, we showed that a further reduction in mucosal pH (from pH 6.1 to 5.2) increased the G_t and the $J_{\text{mannitol-SM}}$ during the recovery period, which equated to approximately 120 to 180 min after initiation of the acidic insult. Collectively, these data indicate increased paracellular permeability after recovery from a severe acidic insult (i.e., decreased barrier function; Schweigel et al., 2005). However, during the ACID challenge itself (approximately 30 to 90 min after addition of the acidic insult), G_t and $J_{\text{mannitol-SM}}$ were not different from that of CON epithelia incubated at mucosal pH 6.1. Similar to our results, Emmanuel et al. (2007) reported that mucosal pH values of 4.5 and 5.5 did not affect the mannitol flux rate during the flux measurement period immediately following the decrease of luminal pH (approximately 0 to 35 min). The delayed onset of a paracellular opening after an acid insult may be explained by a physical occlusion of the paracellular space attributed to acid-induced cell swelling (Gaebel et al., 1989), which initially counteracts the opening of the paracellular space because of tight junctional and cellular damage.

In contrast to the present study, Aschenbach et al. (2000a) observed an increase for the mannitol flux rate immediately after mucosal acidification to a pH of 5.1. The increase in the mannitol flux rate in that study occurred between 30 and 90 min after the initiation of the mucosal acidification (i.e., equivalent to the in vitro challenge period in the current study). The earlier onset of paracellular opening in the study of Aschenbach et al. (2000a) may be a result of a different acidification protocol including the type of acid (i.e., HCl vs. gluconic acid) and the relative reduction in mucosal pH. In the study of Aschenbach et al. (2000a), epithelia were equilibrated to a mucosal pH of 7.4 before a sudden decrease in mucosal pH to 5.1 (a reduction of 2.3 pH units). In comparison, the mucosal side of the epithelia in the current study was equilibrated to a pH of 6.1 before mucosal acidification to a pH of 5.2. Thus, the pH reduction imposed in our study (0.9 pH units) was not as drastic as that imposed by Aschenbach et al. (2000a). Assuming that acid defense mechanisms need some time to function at optimum capacity, the preacidification pH and the rate of acidification are likely important determinants for the degree of acid-induced damage. This may also explain why the mucosal acidification to nadir pH 5.48 was tolerated by ruminal epithelia in vivo because the pH decrease was relatively gradual, with nadir pH being reached 128 min after glucose infusion.

Although it is clear that low mucosal pH consistent with acute ruminal acidosis reduces the net absorption of Na^+ , Cl^- , and Mg^{2+} (Gaebel et al., 1987), the data from the current study do not give indication that preexposure to a mild episode of SARA in vivo had persisting effects on ion transport. The I_{sc} , as a measure for active epithelial ion transfer, as well as G_t , as a measure of passive ion "leakage," were not persistently affected by the in vivo treatment.

The effect of mucosal hyperosmolarity on ruminal epithelial function has been elucidated in past studies demonstrating that alterations in function are attributed to an increase in paracellular permeability (i.e., reduced barrier function) and a reduction in active Na^+ transport (Schweigel et al., 2005; Lodemann and Martens, 2006). The current study indicates that previous exposure to a mild episode of SARA does not alter the response of epithelia to a hyperosmotic challenge. Epithelia from sham and SARA sheep responded similarly to a luminal hyperosmotic challenge with an increase in $J_{\text{mannitol-SM}}$ and G_t . Whereas the increase in $J_{\text{mannitol-SM}}$ may partially be seen as a sequel to osmotic water flow from the serosal to the mucosal side (i.e., solvent drag), the concurrent increase in G_t suggests that an increase in passive permeability likely contributed to the increased $J_{\text{mannitol-SM}}$. As such, our results confirm

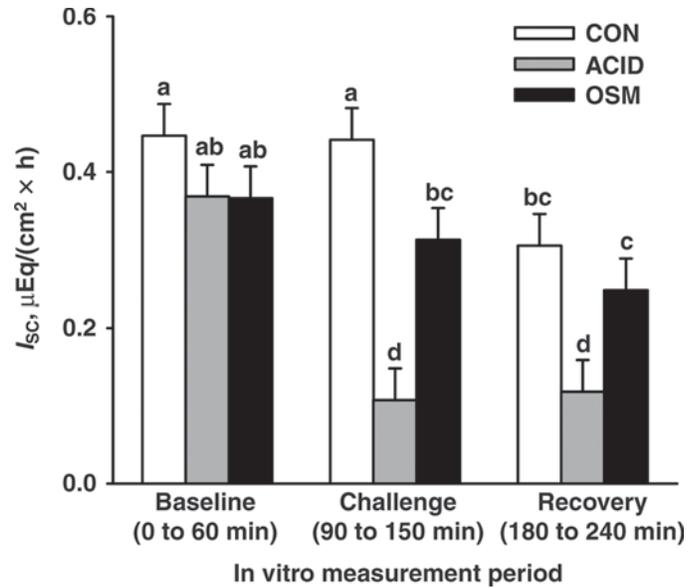


Figure 3. In vitro measurement period \times in vitro treatment interaction for short-circuit current (I_{sc}) in ovine ruminal epithelia ($n = 24$). Epithelia were subjected to three 1-h flux measurement periods consisting of baseline, challenge, and recovery periods that were interspersed by 30-min periods for treatment equilibration. Baseline conditions were pH 6.1 (mucosal solution) and pH 7.4 (serosal solution) with a bilateral osmolarity of 293 mOsm/L. During the challenge period, the mucosal side of the epithelia was exposed to either an acidotic challenge (ACID; pH 5.2, osmolarity 293 mOsm/L) or an osmotic challenge (OSM; pH 6.1, osmolarity 450 mOsm/L); a third group served as control (CON; pH 6.1, osmolarity 293 mOsm/L). The mucosal buffer solution was replaced for the recovery period. Significant effects were detected for the in vitro treatment ($P < 0.001$), in vitro measurement period ($P < 0.001$), and in vitro treatment \times in vitro measurement period ($P < 0.001$). Means with different letters (a, b, c, and d) differ significantly ($P < 0.05$).

previous findings (Schweigel et al., 2005; Lodemann and Martens, 2006) that mucosal hyperosmolarity rapidly, but reversibly, impairs epithelial barrier function with no detectable carry-over effects following the removal of the hyperosmotic challenge. These data imply that hyperosmolarity may contribute to an initial disruption of epithelial barrier function but that the effects are recoverable following return to normal osmotic conditions, at least, as long as hyperosmolarity occurs isolated from other insults.

CONCLUSIONS

Under the conditions imposed, a mild episode of SARA, diagnosed using the area under the pH threshold of 5.8 or a nadir pH value of 5.5, did not negatively affect epithelial barrier function in the short term. However, a rapid and slightly more severe acidification in vitro resulted in a decrease in barrier function as indicated by a greater $J_{\text{mannitol-SM}}$ and G_t approximately 2 h after the insult when mucosal pH had already returned to

pH 6.1. Mucosal hyperosmolarity rapidly, but reversibly, decreased epithelial barrier function. These data suggest that barrier failure during SARA is most likely not a sole sequel of low luminal pH and hyperosmolarity but requires additional insults like parakeratosis and epithelial inflammation. However, if severe enough, low pH alone may be sufficient to induce increases in epithelial permeability. Under this circumstance, the acid-induced barrier failure likely marks the onset of acute ruminal acidosis.

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