Segmental Difference of Mucosal Damage along the Length of a Mouse Small Intestine in an Ussing Chamber

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Summary The Ussing chamber is often used for in vitro investigations into the transport physiology of epithelia including the small intestine. However, the morphological and functional integrity of the small intestine incubated in an Ussing chamber has been largely ignored. The present study attempted to compare the mucosal injury among different segments of the mouse small intestine when incubated in an Ussing chamber. To assess the functional damage, we measured changes in the transmural potential difference (PD) evoked by adding glucose to the mucosal solution and forskolin to the mucosal and serosal solutions. Morphological deterioration was assessed histologically. The villi in the duodenum and proximal jejunum were morphologically damaged by 2 h of incubation in an Ussing chamber and almost completely destroyed within 4 h, while crypts remained intact. The villi were moderately damaged in the distal jejunum. In contrast, the integrity of the villi and crypts was maintained in the ileum for 4 h. The basal PD and forskolin-induced PD were maintained up to 4 h of incubation in all segments. On the other hand, the glucose-induced PD was not apparent in the duodenum at 0 h, and was gradually suppressed to zero in the proximal jejunum by 4 h, although the glucose-induced PD was maintained for 4 h in the ileum. The loss of villous epithelial integrity was correlated with the disappearance of the glucose-induced PD, while both the basal and forskolin-induced PD were retained, even in the tissue with disrupted villi. It is concluded that the mucosa of the proximal small intestine was rapidly injured in the Ussing chamber, particularly in the villi, while the integrity of the mucosa of the distal small intestine was maintained for 4 h. The glucose-induced PD, but not the basal or forskolin-induced PD, can be used as a marker of the villous integrity.

Key Words intestinal absorption, intestinal secretion, intestinal mucosa, villous injury, crypt

The Ussing chamber has been widely used for in vitro studies of transport in a variety of epithelial tissues (1). One of the advantages of this technique is that the electrogenic transport processes of the epithelia can be assessed specifically and precisely with time by simply measuring the transepithelial potential difference (PD). The Ussing chamber has also been applied for transport studies in the small intestine (2, 3). However, most of these studies on the small intestine have largely ignored the tissue integrity, although previous studies have indicated that small intestinal specimens in the chamber exhibited morphological deterioration, particularly of their mucosa (4–7). The fragility of the small intestine in an Ussing chamber may result in the information obtained by this technique being unreliable and controversial.

The mucosal damage in an Ussing chamber just mentioned has not yet been explored in the mouse small intestine. In addition, the segmental difference in mucosal fragility along the length of the small intestine has not been extensively studied. The purpose of this study is, therefore, to characterize the injury to the mouse small intestine in an Ussing chamber. We first histologically compare the mucosal damage that occurred in the Ussing chamber along the length of the small intestine. Then, in order to assess the functional damage, we measure changes in the values of the transmural PD evoked by glucose, which represents the function of the villous epithelium (8), and in the PD derived from Cl− secretion which represents the function of the crypt (9). We finally attempt to correlate the changes in these functional parameters with the histologically determined tissue damage.

METHODS

Tissue preparation and Ussing chamber. Male mice (30–40 g, 7–8 wk old, Std:ddY; Japan SLC, Hamamatsu, Japan) had free access to a conventional diet (MF; Oriental Yeast, Tokyo, Japan) until the day of the experiment. The mice were held for 5–7 d to adapt to the environment before the experiment was started. The mice were then sacrificed by cervical dislocation.

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between 10:00 and 14:00 h, and the entire small intestine from the pylorus to ileocecal junction was excised. The duodenum (D) was the segment from the pylorus to the ligament of Treiz, and the distal ileum (I2) was the 4-cm-long, most aboral segment. The remainder of the small intestine was divided into three segments of equal length comprising the proximal jejunum (J1), distal jejunum (J2) and proximal ileum (I1). The proximal 1-3 cm part of each segment was excised, opened, and washed with an oxygenated buffered solution as described later, before being divided into up to 3 pieces (each about 1 cm in length). Four tissue samples were mounted in Ussing chambers for each animal. Each whole-thickness intestinal piece was stretched and positioned with the mucosal side down on a piece of filter paper that had a hole of 5 mm in diameter, and then mounted between Ussing chambers with an exposed area of 0.2 cm² (5 mm in diameter). The volume of the bathing solution on each side was 5 mL and the solution temperature was maintained at 37°C in a water-jacketed reservoir. Incubation of the tissue in the chamber was started within 5-10 min of the animal being sacrificed. The reason why we chose a whole-thickness tissue rather than a muscle-stripped tissue was to avoid a long preparation time and to start the incubation as soon as possible. All procedures used in this study were performed in accordance with the “Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences” published by the Physiological Society of Japan.

Electrophysiology. The transmural PD was continuously measured with a voltage/current-clamped amplifier (CEZ-9100; Nihon Kohden, Tokyo, Japan) through a pair of calomel electrodes kept in contact with the mucosal and serosal bathing solutions via a pair of 3 M KCl agar bridges. The solution was kept virtually grounded through a pair of Ag/AgCl electrodes kept in contact with the mucosal and serosal bathing solutions via a pair of 1 M NaCl agar bridges. The tissue was incubated for 10 min in the Ussing chamber for stabilization before starting the PD measurements (time 0 h). To determine the glucose-induced PD, 5 mM glucose (final concentration) was added to the mucosal solution at 0, 2 and 4 h. After the glucose-induced PD was measured each time, the mucosal and serosal bathing solutions were replaced by fresh solutions (described later). Forskolin was added to both the mucosal and serosal solutions after adding glucose to determine the cAMP-induced electrogenic Cl⁻ secretion.

Histology. The morphological changes in each segment of the mouse small intestine 2 h and 4 h after starting incubation in the Ussing chamber were examined with an optical microscope. Each tissue sample was removed from the chamber after the incubation, immediately fixed in 4% formaldehyde, embedded in paraffin, sectioned, and stained with haematoxylin/eosin according to the routine method.

Solutions. The composition of the solution was (in mM) NaCl (119), NaHCO₃ (21), KH₂PO₄ (0.6), K₂HPO₄ (2.4), CaCl₂ (1.2) and MgCl₂ (1.2), and was bubbled with 95% O₂/5% CO₂ (pH 7.4). The bathing solution on the mucosal side was supplemented with 8.5 mM mannose, and the serosal solution with 5 mM glucose, 2.5 mM L-glutamine and 1 mM β-hydroxybutyric acid as metabolic substrates.

Statistical analysis. The nonparametric Kruskal-Wallis test was used to test the segmental difference. For a significant difference among segments, a post hoc Dunn's multiple-comparison test was conducted. Fisher's exact test was used for contingency tables. Significance was accepted at p<0.05. All of the comparisons were made using GraphPad Prism version 4.0 (GraphPad Software, San Diego, CA).

RESULTS

1. Histology

The duodenum specimens (D) showed massive sloughing of the villous epithelial cells at 2 h and extensive destruction of villi at 4 h, while the crypts were essentially preserved until 4 h in the Ussing chamber (Figs. 1a, b and c). The upper jejunum (J1) was similar to the duodenum, the villi being extensively damaged at 2 h and almost completely destroyed at 4 h, while the crypts remained intact (Figs. 1d, e and f).

Fig. 1. Morphology of the upper small intestine incubated in an Ussing chamber. Normal tissues (a, d and g), and those incubated for 2 h (b, e and h) and for 4 h (c, f and i) in the Ussing chamber were obtained from the duodenum (D; a, b and c), upper jejunum (J1; d, e and f) and lower jejunum (J2; g, h and i). At least three specimens were examined for each incubation condition. Bars indicate 100 μm.
Table 1. Villous epithelial integrity at 4 h.

| Segment | Intact | Broken |
|---------|--------|--------|
| J1*     | 1      | 6      |
| I2*     | 4      | 0      |

Tissues are grouped according to whether most villi were covered with epithelial cells, although somewhat dysplastic (intact), or whether the villus epithelia were substantially disrupted (broken). The number of tissues for each category is given.

*Significantly different between J1 and I2 by Fisher’s exact test.

2. Electrophysiology

The time-course changes in transmural PD under basal conditions are summarized for each segment of the mouse small intestine incubated for 4 h in the Ussing chamber (Fig. 3). The basal PD at 0 h did not vary among the segments (Fig. 3A). Between 0 and 2 h, the PD declined, although not to zero, and remained at those levels for up to 4 h in all segments (Fig. 3B).

The addition of glucose to the mucosal solution evoked a PD change with increased serosal positivity due to Na+ absorption coupled with glucose absorption (Fig. 4) (8). At 0 h in the Ussing chamber, as summarized in Fig. 5A, 5 mM glucose caused no change in PD in D, a very small, serosa-positive PD increase in J1, and a moderate PD increase in J2 and I1, while it caused a larger PD increase in I2 than in the more proximal intestinal segments (D and J1). We then examined the time-dependent change in glucose-induced PD in each segment of the small intestine during the 4 h of incubation (Fig. 5B). In the duodenum, no glucose-induced PD was apparent during 4 h. In J1, a small glucose-induced PD value was observed in some tissues at 2 h, but was not evident after 4 h of incubation in all the tissues examined. In J2, some tissues showed glucose-induced PD for 4 h, but the others failed to show it by 2 h. However, both ileal segments (I1 and I2) maintained glucose-induced PD during 4 h of incubation in the Ussing chamber. The disappearance of glucose-induced PD by 4 h occurred significantly more frequently in J1 than in I2 (Table 2).

Forskolin, an activator of adenylate cyclase, caused a serosa-positive PD increase to reflect the cAMP-activated electrogenic Cl− secretion (Fig. 4). As summarized in Fig. 6, the magnitude of this forskolin-induced PD was not markedly different along the length of the small intestine. In contrast to the glucose-induced PD, this forskolin-induced PD was apparent in all segments of the small intestine incubated for 4 h in the Ussing chamber.

Histological observation showed that the villous structure deteriorated, depending on the intestinal segment and incubation time, prompting us to correlate covering these shortened villi were flat or cuboidal rather than columnar, as is the case for normal tissue (Fig. 2g). The destruction of the villous epithelium within 4 h occurred significantly more frequently in the J1 than in the I2 specimens (Table 1).
Fig. 3. Basal transmural potential difference (PD) in each segment of the small intestine in the Ussing chamber. A: Comparison of basal PD among the intestinal segments 10 min after starting incubation in the Ussing chamber (0 h). Bars indicate ± SD. B: Time-course characteristics for basal PD in the duodenum (D), upper jejunum (J1), lower jejunum (J2), upper ileum (I1) and lower ileum (I2) while incubating in the Ussing chamber.

Fig. 4. Glucose and forskolin-induced PD. Typical recordings are shown. Zero PD level for each recording is indicated by a dot. A: Two recordings were made for the same tissue obtained from the upper jejunum (J1), and B: the two recordings were made for the same tissue from the lower ileum (I2). Glucose (5 mM) was added to the mucosal solution at the points marked by the arrows. Note that glucose-induced PD in the J1 tissue was detectable at 0 h, but not at 4 h. Forskolin (FK, 5 μM) was added after glucose to both the mucosal and serosal solutions at the points marked by the arrows.
Fig. 5. Glucose-induced PD in each segment of the small intestine incubated in the Ussing chamber. A: Comparison among intestinal segments of the glucose-induced PD 10 min after starting incubation in the Ussing chamber (0 h). Bars indicate ± SD. Segments denoted with different symbols differ significantly from each other. B: Time-course characteristics for glucose-induced PD in the duodenum (D), upper jejunum (J1), lower jejunum (J2), upper ileum (I1) and lower ileum (I2) while incubating in the Ussing chamber. The glucose-induced PD was repeatedly recorded for the same tissue by washing the tissue with a fresh solution between each recording.

Fig. 6. Forskolin-induced PD in each segment of the small intestine incubated in the Ussing chamber. Unlike the basal- and glucose-induced PD, the forskolin-induced PD was determined once for each tissue at the end of incubation, because, unlike the case for glucose, it was not clear whether or not the effect of forskolin (FK) was reversible.
Table 2. Disappearance of the glucose-induced PD at 4 h.

| Segment | Maintained | Disappeared |
|---------|------------|-------------|
| J1*     | 0          | 4           |
| I2*     | 5          | 0           |

The data shown in Fig. 5 have been summarized. The number of tissues for each category is given.

*Significantly different between J1 and I2 by Fisher's exact test.

Fig. 7. Correlation between the disruption of villous epithelial integrity and the electrophysiological parameters. Tissues are grouped according to whether most villi were covered with epithelial cells, although somewhat dysplastic (intact; cf. Fig. 2c), or whether the villus epithelium was substantially disrupted (broken; cf. Fig. 1b). The electrophysiological parameters for each tissue were obtained just before the tissue was removed from the Ussing chamber for the morphological examination. The data obtained for the duodenum were excluded. A: Glucose-induced PD. B: Basal PD. C: Forskolin-induced PD.

Discussion

Our morphological observations show that, like the rat and human small intestine (4–6), the mucosa of the mouse small intestine was damaged, especially in the villi, by in-vitro incubation in the Ussing chamber. In addition, we found that this villous damage was severe in the proximal segments, while villous integrity was maintained for 4 h in the distal segments of the small intestine. Thus, villous destruction was apparent by 2 h in the duodenum (D) and proximal jejunum (J1), while the epithelial layer covered the villi for at least 4 h in the distal small intestine (I1 and I2), although the villi seemed to become shorter and the epithelial cells on the villi became flatter. The villous destruction was moderate in the intermediate segment, i.e. the lower jejunum (J2).

It has been shown that structural deterioration occasionally occurred in the small intestinal preparations used for in vitro studies (10). Soderholm et al. (4) have reported that the human ileal epithelium was lifted from the basal lamina within a period of 90 min of incubation in an Ussing chamber (4). In the rat intestine, destruction to the tip of the villi, an accumulation of cellular debris on the mucosal surface and a reduction in villous height were found (5, 7).

The present study has demonstrated that the mucosal damage in an Ussing chamber occurred in the proximal segments of the small intestine, while epithelial integrity was maintained in the distal segments. Consistent with the present findings, a previous study has demonstrated that the ileum was more resistant to cold preservation than the jejunum (12). In contrast, the rat small intestine has exhibited no marked segmental difference in mucosal damage while in the Ussing chamber (5). In addition, regeneration after the destruction of mucosal integrity by prolonged preservation and transplantation was more pronounced in the jejunum than in the ileum (13). The reason why the proximal small intestine was more fragile than the distal small intestine in the present Ussing chamber exper-
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