Effect of hydrochloric acid, pepsin, or taurocholate on bioelectric properties of gastric squamous mucosa in horses

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Objective—To determine the effect of pH with or without pepsin or taurocholic acid on the bioelectric properties of gastric squamous mucosa in horses.

Sample Population—Gastric tissues obtained from 16 adult horses that did not have evidence of gastric disease.

Procedure—Bioelectric properties of squamous mucosa were determined, using modified Ussing chambers. Tissues then were exposed to mucosal pepsin (1 mg/ml) or taurocholic acid (2.5 mM) under neutral (pH 7.4) or acidic (pH 1.7) conditions.

Results—Exposure of mucosal sheets to an acidic pH resulted in an immediate and sustained decrease in transmembrane potential difference and calculated tissue resistance. Pepsin or taurocholic acid did not significantly affect bioelectric variables when added to a mucosal bath solution of pH 7.4. A synergistic effect between pepsin or taurocholic acid and mucosal acidification was not detected.

Conclusions and Clinical Relevance—Mucosal acidification with or without pepsin or taurocholic acid resulted in reduced tissue resistance. These data support the contention that squamous erosions or ulcers in horses are mediated, in part, by prolonged exposure of gastric squamous mucosa to luminal acid.

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tory simple columnar epithelium of the cardiac gland region at the margo plicatus. Ulcers, hyperkeratosis, and erosions are frequently located at this junctional region, particularly on the lesser curvature of the stomach, because this anatomic location is likely exposure to prolonged periods of acidity as well as duodenal contents that reflux through the pyloric sphincter (enterogastric reflux).

The specific objectives of the study reported here were to determine the bioelectric properties of short-circuit current ($I_{SC}$), potential difference (PD), and resistance (R) in isolated equine gastric squamous mucosa and to characterize specific responses to modulators of ion transport (ie, barium chloride, acetazolamide, amiloride, bumetanide, and ouabain). We also examined the effects of luminal acid on these bioelectric properties and evaluated the effect on those properties attributable to pepsin or a conjugated bile salt (taurocholate) in neutral or acidic conditions.

**Materials and Methods**

**Collection of tissues**—Tissue specimens were obtained from 16 adult horses (>1 year old) that were euthanatized for reasons unrelated to disease of the gastrointestinal tract. None of the horses had received any medication during the 24-hour period preceding euthanasia. Immediately after IV injection of an overdose of barbiturate, the stomach was removed from each horse and incised through the cardia and extending along the greater curvature. Gastric mucosa was not collected when there was evidence of recent or active ulcers or when >5 Gastrophilus spp larvae were detected. A full-thickness section of gastric wall lined with stratified squamous epithelium was harvested (10 × 30 cm) from an area approximately 1 cm from the margo plicatus, rinsed to remove ingesta, and placed in Ringer's solution (24°C) for transport to the laboratory.

Tissues were pinned with the mucosal side facing down in a paraffin dissecting tray and continuously bathed with Ringer's solution. Squamous mucosa was removed from muscle layers and submucosa by sharp dissection, rinsed in Ringer's solution, and mounted in modified Ussing chambers with an aperture of 1.327 cm². Tissues were bathed with 10 ml of Ringer's solution on the mucosal and serosal surfaces, and a gas mixture (99% O₂ and 1% CO₂) was continuously bubbled through the solution, using a circulating bubble lift. The water-jacketed reservoir was maintained at 37°C. Tissues were allowed to stabilize for 30 minutes in normal bicarbonate-buffered Ringer's solution (pH 7.4), and the tissues were monitored for an additional 60 minutes.

**Characterization of tissues**—Techniques used to examine basal secretion and absorption of tissues included the addition of mucosal amiloride (10 µM of 10⁻³ M), final concentration, 10⁻³ M; serosal bumetanide (20 µM of 10⁻³ M), final concentration, 2 X 10⁻³ M; serosal or mucosal ouabain (10 µM of 5 X 10⁻³ M), final concentration, 5 X 10⁻³ M), and replacement of bicarbonate with N-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid (HEPES) in conjunction with serosal acetazolamide (50 µM of 10⁻³ M); final concentration, 5 X 10⁻³ M). The effect of serosal barium chloride (500 µl of 10⁻³ M; final concentration, 5 X 10⁻³ M) also was evaluated at pH 7.4 and pH 1.7.

**Results**

**Results for untreated tissues**—Mean ± SEM PD was 19.75 ± 1.35 mV and varied little with time in tissues maintained at pH 7.4. Mean $I_{SC}$ tended to decrease slowly throughout the experiment from an initial value of −11.68 ± 1.3 µA/cm² to a value of −7.45 ± 0.78 µA/cm² at 150 minutes. Consequently, calculated value for tissue R increased over the same period from an initial value of 1.798 ± 161 to 2.847 ± 312 Ω/cm².

**Effects of ion transport modulators**—Bioelectric variables were not significantly affected by the mucosal addition of amiloride, a transmembrane Na⁺-channel inhibitor, the serosal addition of bumetanide, a Na⁺-K⁺-Cl⁻-transporter inhibitor, the mucosal addition of ouabain, a Na⁺-K⁺-ATPase inhibitor, or replacement of bicarbonate with HEPES and the addition of mucosal acetazolamide, a carbonic anhydrase inhibitor. Addition of ouabain to the serosal bath solution reduced mean PD from 15.77 ± 2.4 to 10.37 ± 1.63 mV and reduced mean $I_{SC}$ from −7.86 ± 1.21 to −4.95 ± 0.76 µA/cm²; however, it did not have a significant effect on calculated R. Addition of barium chloride to the serosal bath solution but not the mucosal bath solution resulted in a sudden and sustained large decrease in PD (17.1 ± 3.8 to 9.28 ± 2.1 mV; Fig 1) and $I_{SC}$ (−13.6 ± 3.0 to −5.09 ± 1.1 µA/cm²; Fig 2). There was not a significant change
in calculated R after the addition of barium chloride to the serosal bath solution.

Effect of acidification—Acidification of bath solutions resulted in a decrease in PD (21.5 ± 2.2 to 0.66 ± 1.5 mV; Fig 3) and $I_{SC}$ (−11.8 ± 2.1 to +4.4 ± 5.0 µA/cm²; Fig 4) during the period of acid exposure. A gradual but continuous decrease in PD was detected throughout the acid-exposure portion of the experiment. Following removal of acid, mean PD increased to 11.6 ± 2.0 mV, a value significantly less than the value obtained for the tissues before exposure to acid and the value for control tissues (pH 7.4) at 180 minutes (18.7 ± 1.2 mV; Table 1). After the acidic bath solution was replaced with Ringer’s solution (pH 7.4), the $I_{SC}$ (−7.3 ± 0.9 µA/cm²) recovered to approximately the same magnitude as for control tissues (−7.3 ± 0.9 µA/cm²). Tissue R declined steeply during the initial 30 minutes of acid exposure (from a starting value of 2,014 ± 173 to 1,675 ± 179 Ω/cm²), then decreased slowly over the remaining period to achieve a final value of 1,026 ± 179 Ω/cm². After the acidic bath solution was replaced with Ringer’s solution (pH 7.4), the R of

<table>
<thead>
<tr>
<th>PD (mV)</th>
<th>Before treatment (t=0 min)</th>
<th>During treatment (t=150 min)</th>
<th>After treatment (t=30 min recovery)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 7.4</td>
<td>19.75 ± 1.50</td>
<td>19.84 ± 1.10</td>
<td>18.73 ± 1.20</td>
</tr>
<tr>
<td>pH 1.7</td>
<td>21.5 ± 2.19</td>
<td>0.66 ± 1.48</td>
<td>11.58 ± 2.05</td>
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<tr>
<td>pH 7.4 + pepsin*</td>
<td>20.45 ± 2.21</td>
<td>21.47 ± 1.95</td>
<td>18.51 ± 1.98</td>
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<tr>
<td>pH 1.7 + pepsin*</td>
<td>18.75 ± 1.74</td>
<td>0.19 ± 1.13</td>
<td>10.68 ± 1.59</td>
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<tr>
<td>pH 7.4 + bile salt†</td>
<td>20.46 ± 3.02</td>
<td>18.08 ± 2.67</td>
<td>16.66 ± 2.71</td>
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<tr>
<td>pH 1.7 + bile salt†</td>
<td>19.98 ± 1.51</td>
<td>−0.38 ± 1.27</td>
<td>10.13 ± 1.22</td>
</tr>
</tbody>
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Table 1—Effect of acidic conditions with or without pepsin or bile salt in bath solutions on mean ± SEM transmembrane potential difference (PD) for equine gastric mucosa

Figure 1—Mean ± SEM values for transmembrane potential difference of equine gastric mucosa exposed to a bath solution of Ringer’s solution, pH 7.4 (control; black circle); Ringer’s solution, pH 1.7, and 500 µl of 10⁻¹ M barium chloride (BaCl₂ only; white square); or Ringer’s solution, pH 1.7, and 500 µl of 10⁻¹ M barium chloride (BaCl₂ and acid; black triangle). Measurements were obtained before addition of barium chloride to the bath solution. Acidic bath solutions were initiated at time 0. Acidic bath solution was replaced with pH-neutral solution 150 minutes later. Measurements were obtained 30 minutes after return to pH-neutral solution (ie, 30 minutes of recovery [30R]).

Figure 2—Mean ± SEM values for short-circuit current of equine gastric mucosa exposed to various bath solutions. See Figure 1 for key.

Figure 3—Mean ± SEM transmembrane potential difference of equine gastric mucosa exposed to bath solutions of pH 7.4 or 1.7.

Figure 4—Mean ± SEM short-circuit current of equine gastric mucosa exposed to bath solutions of pH 7.4 or 1.7.

Table 1—Effect of acidic conditions with or without pepsin or bile salt in bath solutions on mean ± SEM transmembrane potential difference (PD) for equine gastric mucosa
Table 2—Effect of acidic conditions with or without pepsin or bile salt in bath solutions on mean ± SEM calculated tissue resistance (R) for equine gastric mucosa

<table>
<thead>
<tr>
<th>Before treatment</th>
<th>During treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>t=0 min</td>
<td>t=150 min</td>
</tr>
<tr>
<td>pH 7.4</td>
<td>1,798 ± 161</td>
<td>2,847 ± 312</td>
</tr>
<tr>
<td>pH 1.7</td>
<td>2,014 ± 179</td>
<td>1,026 ± 179</td>
</tr>
<tr>
<td>pH 1.7 + pepsin*</td>
<td>2,130 ± 264</td>
<td>1,135 ± 316</td>
</tr>
<tr>
<td>pH 7.4 + bile salt</td>
<td>1,544 ± 202</td>
<td>2,579 ± 433</td>
</tr>
<tr>
<td>pH 7.4 + bile salt</td>
<td>2,033 ± 334</td>
<td>1,023 ± 348</td>
</tr>
</tbody>
</table>

See Table 1 for key.

Acid-treated tissues did not approach that of control tissue values after 30 minutes of recovery (1,610 vs 2,789 Ω/cm²; Table 2).

Effect of acidification and pepsin—A bath solution of pH 1.7 with the addition of pepsin (1 mg/ml) resulted in a decrease of PD from an initial value of 18.75 ± 1.74 to 0.19 ± 1.13 mV after 150 minutes of exposure (Table 1). Mean I sc decreased from –0.98 ± 1.49 to –0.32 ± 1.46 µA/cm² with variable response between individual tissues. Calculated R decreased from 2,130 ± 264 to 1,135 ± 317 Ω/cm² at 150 minutes after initiation of acid and pepsin exposure, but it rebounded after removal of acid (1,587 ± 141 Ω/cm² after 30 minutes of recovery; Table 2).

Effect of acidification and taurocholate—Mean PD decreased from 20.0 ± 1.5 to –0.38 ± 1.3 mV at 150 minutes and failed to return to values similar to those of the control tissues after 30 minutes of recovery at pH 7.4 (Table 1). Initial mean I sc was –11.87 ± 1.88 µA/cm², which steadily decreased to +1.09 ± 2.64 µA/cm² after 150 minutes of acid exposure. Calculated R also decreased from an initial value of 2,033 ± 333 to 1,023 ± 348 Ω/cm² after 150 minutes of acid exposure. This value then increased negligibly after replacement of the bath solution with Ringer’s solution (pH 7.4; Table 2).

Effect of pepsin—We did not detect significant differences between tissues maintained at pH 7.4 with pepsin and those in bath solutions at pH 7.4 without pepsin.

Effect of taurocholate—We did not detect significant differences between tissues maintained at pH 7.4 with taurocholate and those in bath solutions at pH 7.4 without taurocholate.

Effect of acidification and barium chloride—The magnitude and rate of decrease of PD (Fig 1) and I sc (Fig 2) after acidification were greater in serosal tissues when the tissues had received prior treatment with barium chloride.

Discussion

Resting bioelectric properties of untreated equine gastric squamous epithelium reported here are comparable to those of esophageal squamous epithelium from rabbits and pars esophageal squamous mucosa from pigs. High inherent electrical resistance is a character-
inherently resistant to luminal acid, primarily because of the thickness of the stratum corneum combined with its high electrical resistance and tight epithelial junctions. Cellular ion-transport properties also are likely to be important in controlling intracellular pH, which was documented by treatment of the serosal bath solution with barium chloride prior to acidification of the bath solution. Prolonged exposure to a bath solution with a pH of 1.7 had a damaging effect on gastric squamous epithelium, as measured by PD and calculated R. There was a sudden decrease in values for measured variables followed by a more sustained decrease during the period of acid exposure. After removal of acid, tissues had reduced R, compared with values for control mucosal tissues, indicating sustained damage. The effect of mucosal acidification on equine gastric squamous mucosa was greater than that reported by others; however, the site of tissue collection was not described in detail in that report and may have differed from the site selected for our study. Notably, the principal difference between these 2 studies was the pH of the bath solution; we used a solution with pH 1.7, whereas Berschneider et al used a solution with pH 2.5. Both are within the range of physiologic pH described for equine gastric fluid.

Acid-induced bioelectric changes consistent with tissue damage were not induced in porcine squamous mucosa until mucosal pH was < 1.5. Esophageal mucosa of rabbits was resistant to mucosal acidification at pH 3.0 but damaged when pH was lowered to 1.5. The proposed mechanism for acid-induced injury of squamous mucosa involves an initial increase in mucosal permeability (paracellularly or directly through the cell) followed by inhibition of active sodium transport and loss of osmolar regulatory abilities. Swelling of epithelial cells leads to vesicle formation and sloughing of overlying mucosal layers with exposure of underlying interstitial tissue that is vulnerable to perpetuated acid-induced injury.

The addition of pepsin or taurocholate to the bath solution maintained at pH 7.4 did not have an effect on any of the measured variables. The concentration of pepsin used in this study was determined on the basis of concentrations in gastric fluid obtained from cannulated horses. Pepsinogen, an inactive protease precursor to pepsin, also has been isolated from equine gastric mucosa. Conversion to pepsin is a pH-dependent process, with peak conversion at pH 2.0. Proteolytic activity of the enzyme is also greatest when luminal pH is < 3.0. Consequently, changes in mucosal bioelectric properties would not be expected when the enzyme is added to a fluid of pH 7.4. In contrast to studies that used esophageal tissues obtained from rabbits, dogs, or cats, we were unable to document a synergistic effect of pepsin when added to a bath solution of pH 1.7. It is possible that the pH used in our study was too severe for this tissue type to enable us to identify subtle changes induced by pepsin. Alternatively, the concentration of pepsin used may have been inadequate with respect to physiologic conditions.

Entero gastric reflux of small intestinal contents, including bile salts, has been documented in horses. Total concentration of bile salts ranging from 21 to 77 µmol/L has been reported in gastric fluid from horses from which food has been withheld. In another study, bile salt concentrations < 200 µmol/L were reported in fed horses, with mean concentrations between 225 and 440 µmol/L in horses from which food was withheld. Taurocholate, a taurine conjugate of cholate, is hepatically synthesized and secreted into bile. Conjugated bile salts have a lower pKa than unconjugated salts. The pKa of conjugated taurocholate and taurodeoxycholate is 1.9, whereas the pKs of unconjugated cholate is 3, and the pKa of unconjugated deoxycholate is 3.0. At a pH near its pKa, approximately 50% of a weak acid will be ionized and soluble, and as the pH decreases below the pKa (such as in the gastric environment), more of the compound becomes nonionized, insoluble, and lipophilic, potentially allowing entry into epithelial cells. Therefore, when taurocholate is added to a solution with high pH, it becomes predominately ionized and not easily absorbed, and similar to the situation for pepsin, an effect at pH 7.4 was not anticipated nor observed. Based on its pKa and on limited data from horses and other species, we predicted a synergistic effect of taurocholate in a bath solution with low pH. Unfortunately, as stated previously, the low pH selected in the study reported here may have overshadowed any effect of the bile acid.

Acidic pH has a substantial in vitro corrosive effect on gastric squamous mucosa of horses. Prolonged exposure of mucosa to acidic luminal contents may be a critical factor in the development of squamous erosions and ulcers in horses.

References


