Effects of Ammonia on the Gastric Mucosal Barrier in Rats and Dogs

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Abstract—We examined the effect of ammonia on the gastric mucosal barrier by measuring the changes in transmucosal fluxes of \( H^+ \), \( Na^+ \) and \( K^+ \). In rats, ammonia at concentrations of 0.1 to 0.5% increased the \( H^+ \) loss from the lumen and 0.2 to 0.5% concentrations of ammonia increased both \( Na^+ \) and \( K^+ \) influxes into the lumen. In dogs, in an exactly similar manner to rats, ammonia at concentrations of 0.1 to 0.5% increased \( H^+ \) loss, and ammonia at concentration of 0.5% increased both \( Na^+ \) and \( K^+ \) influxes into the lumen. These results suggest that ammonia breaks the gastric mucosal barrier.

Although gastric and duodenal ulcer are prevalent disorders, their etiology and pathogenesis are not fully understood. It is currently thought that the development of peptic ulceration is related to a disturbance in the balance of acid production and mucosal resistance (1). Recently, accumulated evidence has indicated that species of gastric spiral bacteria, now named *Campylobacter pylori* (C.P.), possesses high urease activity and is associated with gastritis and peptic ulcer (2–8). Our recent studies demonstrated that even a small amount of urea is capable of damaging the gastric mucosa after its conversion to ammonia by urease (9, 10). In the present study, we examined the effect of ammonia on the gastric mucosal barrier by measuring the changes in transmucosal fluxes of \( H^+ \), \( Na^+ \) and \( K^+ \).

Male Sprague Dawley rats (180–200 g in body weight) and male beagle dogs (LR strain, 9–10 kg in body weight), each with a Heidenhain pouch, were used. Both rats and dogs were deprived of food but allowed free access to water prior to the experiments. Rats were anesthetized with urethane (1.25 g/kg, i.p.). The abdomen was incised and the stomach was exposed. An acute gastric fistula was prepared by placing a polyethylene tube in the forestomach and led to a three way tap with a ligature around the neck of the esophagus and pylorus. Acid secretion was completely inhibited by subcutaneous injection of omeprazole (30 mg/kg, Hassle). One milliliter of ammonia (\( \text{NH}_4\text{OH}, 0.1–0.5% \)) was instilled into the stomach. Fifteen minutes later, the gastric content was aspirated and discarded. After the stomach was washed 3 times, 2 ml of acid solution (100 mM HCl and 54 mM NaCl) was instilled for 15 min; then the sample was aspirated and used for analysis. In the study on the dogs, a 10-ml aliquot of ammonia (\( \text{NH}_4\text{OH}, 0.05–0.5% \)) was instilled into the pouch. Fifteen minutes later, the gastric content was aspirated and discarded. After the pouch was washed 3 times, 10 ml of acid solution (100 mM HCl, 15 mM NaCl and 78 mM mannitol) was instilled into the pouch for 30 min; then the sample was aspirated and used for analysis. The concentrations of \( H^+ \) were determined by titration to pH 7.0 with 0.1 N NaOH using an automatic titrator. The concentrations of \( Na^+ \) and \( K^+ \) were determined using a flame photometer. Statistical analysis was performed using Dunnet’s multiple comparison test.

The results of the examination in rats are summarized in Table 1. Ammonia at a concentration of 0.1% significantly increased \( H^+ \)
Table 1. Effect of ammonia on gastric mucosal barrier in fistula rats

| Net ion flux in luminal solution (μeq/15 min) | Concentration of ammonia (%) |
|---------------------------------------------|-----------------------------|
|                                             | 0 (D.W.)  | 0.1   | 0.2   | 0.5   |
| H+ loss a                                   | 18.5±1.9  | 28.7±1.9** | 50.9±3.6*** | 80.5±12.2** |
|                                             | (15.5±1.9) |       |       |       |
| Na+ gain b                                  | 9.5±1.0   | 9.5±1.7   | 27.6±2.5**  | 26.7±4.0**  |
|                                             | (8.7±0.5)  |       |       |       |
| K+ gain c                                   | 0.78±0.07 | 0.87±0.13 | 1.68±0.1*** | 2.11±0.2**  |
|                                             | (0.80±0.02) |       |       |       |

a A loss of titratable acidity from the luminal solution. b, c Net Na+ and K+ flux in luminal solution. D.W. = distilled water. Each value represents the mean±S.E. of 6 animals. **Significantly different from the control (D.W.) at P<0.01. ***Significantly different from the control (D.W.) at P<0.005. ( ) shows ion fluxes before exposure to ammonia.

Table 2. Effect of ammonia on gastric mucosal barrier in dogs with Heidenhain pouch

| Net ion flux in luminal solution (μeq/30 min) | Concentration of ammonia (%) |
|---------------------------------------------|-----------------------------|
|                                             | 0 (D.W.)  | 0.05 | 0.1  | 0.5  |
| H+ loss a                                   | 36.0±24.0  | 92.0±22.0 | 180.0±28.0** | 566.0±60.0** |
|                                             | (25.0±9.0)  |       |       |       |
| Na+ gain b                                  | 101.0±12.0 | 65.0±13.0 | 78.0±15.0  | 713.0±18.0*** |
|                                             | (100.0±10.0) |       |       |       |
| K+ gain c                                   | 11.0±3.0   | 5.0±2.0   | 5.0±0.4   | 29.0±4.0**  |
|                                             | (9.0±2.0)   |       |       |       |

a A loss of titratable acidity from the luminal solution. b, c Net Na+ and K+ flux in luminal solution. D.W. = distilled water. Each value represents the mean±S.E. of 3 animals. **Significantly different from the control (D.W.) at P<0.01. ***Significantly different from the control (D.W.) at P<0.005. ( ) shows ion fluxes before exposure to ammonia.

Loss from the lumen (P<0.01), while the net Na+ and K+ fluxes into the lumen were not changed as compared to distilled water alone. Ammonia at a concentration of 0.2% significantly increased H+ loss from the lumen (P<0.005), which was consistent with the net Na+ and K+ fluxes into the lumen (P<0.01, P<0.005). In a similar manner, exposure to 0.5% ammonia resulted in significant increase of H+ loss from the lumen and resulted in net Na+ and K+ fluxes into the lumen (P<0.01, P<0.005). In the above experiments, after the exposure to ammonia, the gastric lesion was not seen and the volume of test solution was not changed. In the study on dogs (Table 2), although ammonia at a concentration of 0.05% did not affect H+ or the net Na+ or K+ fluxes, exposure to 0.1% ammonia resulted in a significant increase of only H+ loss from

The mechanism of production of ammonia by the action of urease on urea in gastric tissue, and they pointed out the possible role of this mechanism in neutralizing gastric acid by ammonia. Watanabe et al. (12) suggested that the urea-urease-ammonia system is one of the important mechanisms for gastric mucosal protection. On the other hand, this study demonstrated that ammonia at concentrations of 0.1% or above increased H+ loss,
Na⁺ gain and K⁺ gain, indicating the breaking of the gastric mucosal barrier in rats and dogs. Furthermore, it has been demonstrated that ammonia decreased transmucosal potential difference and produced microscopic and macroscopic gastric lesions (9, 10). From these facts as described above, ammonia must be considered as a barrier breaker. It has been recently reported that 15 to 30% of the circulating urea is converted to ammonia by gastrointestinal urease from bacteria in humans (13) and that C.P. is the strain of bacteria responsible for this (3). In the case of infected patients with C.P., the concentration of ammonia in the gastric juice was increased to 0.2% (3). In animal studies, we previously reported that a small amount of urea is capable of damaging the gastric mucosa after conversion to ammonia by urease (9, 10). In conclusion, the present study demonstrated that ammonia acts on the gastric mucosa as a barrier breaker, and it is assumed that ammonia is one of the aggressive factors rather than defensive factors, and a urea-urease-ammonia system is involved in the pathogenesis of gastritis and/or gastric ulcer disease.

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