EFFECT OF SODIUM FLUORIDE ON THE TRANSMURAL POTENTIAL DIFFERENCE OF THE RAT STOMACH

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SUMMARY: Transmural potential difference (PD) was measured in vivo under continuous perfusion of the rat stomach. Luminal NaF (5 mM) perfusion elicited a net temporal decrease of PD, whereas lower NaF concentrations (1 and 2 mM) had no effect. The fall of PD induced by 5 mM NaF was abolished at pH 8.4 and was enhanced (161%) at pH 3.2. Compared to NaF alone, the decrease of PD induced by the simultaneous perfusion of NaF and salicylic acid (5 mM each) was more pronounced (209%) with a previous perfusion of salicylic acid alone than the direct perfusion of NaF and salicylic acid (136%). These results suggest that the observed variations of PD reflect the early functional alterations of the mucosa preceding the structural damaging effects of hydrofluoric and salicylic acids.

Key words: NaF; pH; Potential difference; Rat; Salicylic acid; Stomach.

Introduction

The major pathway by which fluoride enters the circulation is by absorption from the gastrointestinal tract.1-3 Fluoride can be absorbed in appreciable amounts from the stomach due to the more lipid hydrofluoric acid (HF) formation.4,5 Acute and chronic studies in animals and human have shown that NaF causes gastrointestinal damage.6-13 The toxic effects of F\(^-\) and H\(^+\) on enzyme systems and structural damage were attributed to the rapid penetration of HF into cells.7,14,15 Fluoride acting as a proton ionophore can overcome the gastric mucosal barrier, a term accounting for the relative impermeability of gastric epithelium to passive movements of ions.16 The integrity of this diffusion barrier characterized by a high transmucosal potential difference (PD) and low conductance can be monitored with PD measurements.17

The aim of the present study is to examine the effect of NaF on PD and its behaviour as a barrier-breaking agent.

Material and Methods

Male Wistar rats weighing between 200 and 250 g were purchased from Institut Pasteur (Alger, Algeria). The animals were maintained on standard laboratory chow and tap water ad libitum. They were starved for 24 hours in cages with mesh bottoms to minimise coprophagy but allowed free access to drinking water. The rats were anesthetized with an intraperitoneal injection of urethane (1.5 g.Kg\(^{-1}\)).

Operative technique

After a tracheostomy was performed, a tracheal cannula was inserted to ensure free airways. Stomach perfusion was performed by inserting a polyethylene tubing (OD 1.78 mm, ID 1.02 mm) through the oesophagus into the gastric lumen. The abdomen was opened by a midline incision, the pyloro-duodenal junction exposed and a blind glass cannula (OD 5.0 mm, ID 3.5 mm) with lateral holes introduced through a cut in the duodenum into the stomach. The cannula was secured firmly by tying ligation around the pylorus, care being taken to avoid ligating blood vessels. The whole stomach was then brought forward, the wound covered with a moistened cotton wool pad. The animal was maintained along the experiment under a heating lamp.
Continuous recording of transmucosal PD

The transmucosal PD was recorded between the gastric lumen and the peritoneal cavity with a potentiometric recorder (LKB Bromma, Sweden) via matched calomel half-cells (Tacussel, Lyon, France). The half-cells were connected by means of agar bridges (5% agar in 3 M KCl) with their distal ends in the gastric lumen and the peritoneal cavity, respectively. The luminal bridge was in fact introduced into the glass cannula through a small hole in the side of a soft rubber tubing attached to the cannula which served also for drainage of the perfused solution. Asymmetry between the two ends of the measuring system (≤ 0.7 mV) was determined by placing distal ends of the agar bridges in 0.9% NaCl. Potential difference was expressed as luminal face negative with respect to the serosal side. After completion of the operative procedures, the stomach was perfused with prewarmed isotonic solution (37°C) at a rate of 0.8 mL min⁻¹ by means of a peristaltic pump (LKB Bromma, Sweden). All the tested solutions were made isotonic by addition of NaCl.

Experimental protocols

To assess the stability of PD measurements, the stomach was perfused with 0.9% NaCl and PD recorded for 90 min after completion of abdominal surgery.

In order to study the effect of varying concentrations of NaF on PD, perfusion of the stomach was started with 0.9% NaCl until stabilisation of PD (basal PD). This solution was then replaced with isotonic saline containing 1, 2 or 5 mM NaF.

The influence of luminal pH on NaF-induced variations of PD was investigated at pH 3.2 and 8.4. After stabilisation of PD under isotonic NaCl perfusion, the stomach was perfused for 90 min with a test solution containing 5 mM NaF and either 3 mM HCl or 20 mM NaHCO₃.

A fourth experiment was undertaken in order to investigate the influence of salicylic acid (SA) on NaF-induced variations of PD. After stabilisation of PD under isotonic NaCl perfusion, the stomach was perfused for 30 min with isotonic solution containing 5 mM NaF and 5 mM SA just after 30 min perfusion of 0.9% NaCl or of 5 mM salicylic acid. To test the recovery of PD, the test solution was replaced with isotonic NaCl for a further 30 min period.

Statistical calculations: All data are expressed as means ± SE with the number of experiments between brackets. The significance of differences was evaluated by Student’s t test for paired or unpaired data and probability values lower than 0.05 were considered statistically significant.

Results

The perfused stomach developed a PD, serosal side positive. Within 5 min after starting perfusion of isotonic NaCl, the average PD was 31.4 ± 2.2 mV, and subsequently stabilised at 35.0 ± 0.7 mV (48 experiments). Perfusion of the stomach with isotonic NaCl for 90 min resulted in a stable PD. In the subsequent studies, the experiments were undertaken with at least a 30 min period for stabilization. Since PD was not affected by the time course of perfusion, it was not necessary to correct the variations of PD induced by the test solutions.

Figure 1 shows that perfusion of the stomach with 1 or 2 mM NaF was without effect on PD (P > 0.05). But 5 mM NaF led to a temporal decrease of PD (Figure 1). Basal PD fell significantly from 36.5 ± 1.5 to 26.5 ± 1.6 (8) mV at time 25 minutes
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(P < 0.05). Thereafter, PD increased slowly to attain a value not significantly different from the basal value.

The perfusion of the stomach with 5 mM NaF at pH 8.4 (20 mM NaHCO$\text{$_3$}$) had no significant effect on PD (Figure 2). When the luminal pH was lowered to 3.2 by addition of 3 mM HCl, 5 mM NaF caused a net decrease (P < 0.05) of PD from $37.7 \pm 1.7$ to $16.4 \pm 1.4$ (8) mV (Figure 2). Thereafter, PD began to increase. After 90 min, PD had not fully recovered and was significantly decreased compared with basal value.

Perfusion of 5 mM NaF simultaneously with 5 mM SA reduced significantly the PD from $32.7 \pm 1.8$ to $12.4 \pm 0.9$ (8) mV (Figure 3). The ease of PD was partially reversible since the replacement of the test solution with isotonic NaCl rapidly increased PD but without reaching the basal value (P < 0.05). After 30 min, PD was 18.3% lower than the basal value. Perfusion of 5 mM SA alone was able to induce a decrease of PD from $38.8 \pm 1.8$ to $21.1 \pm 2.5$ (8) mV (Figure 4). When 5 mM NaF was added to the medium, a further decrease of PD to $13.5 \pm 2.4$ (8) mV was observed. The replacement of the previous solution with NaCl was able to increase PD but without reaching the basal value (P < 0.05). After 30 min perfusion of NaCl, PD was 19.6% lower than the basal value.

Figure 5 shows comparisons between treatments of the maximal decrease of PD ($\Delta$ PD). Addition of 3 mM HCl or 5 mM SA to the NaF solution (5 mM) enhanced $\Delta$ DPmax by 161% and 136% respectively. Under perfusion of the stomach with SA and then with SA plus NaF, $\Delta$ DPmax was successively 116% and 209% greater than that induced by NaF alone.

Figure 1. Effects of luminal perfusion of various concentrations of NaF on gastric transmural PD. Upper panel: 1 mM (○), 2 mM (●); lower panel: 5 mM. Vertical lines represent one SE (n = 8). * Significant difference from basal value (B).
Figure 2. Effects of luminal perfusion of 5 mM NaF at pH 3.2 (●) and 8.4 (●) on gastric transmural PD. Vertical lines represent one SE (n = 8). * Significant difference from basal value (B).

Figure 3. Effect of luminal perfusion of 5 mM NaF and 5 mM salicylic acid on gastric transmural PD. Vertical lines represent one SE (n=8). * Significant difference from basal value (B).
Figure 4. Effect of luminal perfusion of 5 mM NaF and 5 mM salicylic acid on gastric transmural PD following a 30 min perfusion of salicylic acid alone. Vertical lines represent one SE (n=6). * Significant difference from basal value (B).

Figure 5. Maximal variations of PD induced by perfusion of: 1) NaF; 2) NaF + 3 mM HCl; 3) salicylic acid; 4) salicylic acid + NaF just after isotonic NaCl; 5) salicylic acid + NaF just after salicylic acid. NaF and salicylic acid concentrations were set to 5 mM. Vertical lines represent one SE. * Significantly different from 1), unless otherwise indicated.
Discussion

The gastric preparation used in this study showed that NaF was able to change the electrical properties of the mucosa. The extent and reversibility of the fall of PD induced by NaF were dependant upon concentration, luminal pH, and time of exposure of the stomach to SA.

A temporal decrease of PD was observed with 5 mM NaF but not with lower concentrations. The decrease of PD was 2.6 times at pH 3.2 and completely abolished at pH 8.4. Fluoride is a weak acid with a pKa of 3.5. At pH 3.2, 67% of fluoride exists as unionized hydrofluoric acid (HF), the main form of passive transport by epithelial cells.45,18-20 Once inside the cell (pH 7.4), the HF is buffered by intracellular proteins and rapidly dissociates releasing the fluoride anion.21 The pH-dependant decrease of PD seems resulting from the increasing amounts of F- and H+ entering the cell interior. Similarly, SA reduced the PD, except that it is twice more potent than NaF at the same concentration. Salicylic acid belongs to the category of the barrier-breaking agents that act synergistically to enhance the noxious effects of H+.22,23 It was shown that acetylsalicylic acid (10-20 mM) induced a marked fall of PD and electrical resistance of frog gastric mucosa preparation.24,25 Furthermore, oral administration of aspirin to humans resulted in a drastic fall of the gastric transepithelial PD.26 The actual enhanced ΔPDmax (30%) observed following pre-exposure of the stomach to SA may result from the decrease of the electrical resistance, rendering the mucosa more permeable to fluoride.

Acute and chronic studies in animals and human have shown that NaF causes extensive gastroduodenal mucosal damage: petechiae, erosion, erythema, denudation of the mucosa, and degeneration of the epithelial cells.6-13 The toxic effects of F- and H+ on enzyme systems and structural damage were attributed to rapid penetration HF across lipid cell membranes.7,14,15

The present study suggests that the decrease of PD induced by relatively low concentrations of NaF may reflect the early functional modifications of the gastric mucosa before the appearance of structural injury. These changes may be altered by manipulation of the luminal environment and the epithelial resistance.

References

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