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Kinetics of Acetylsalicylate and D-lactate Transport Across Isolated Frog Gastric Mucosa

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ABSTRACT

Luminal to submucosal migration of ^{14}C-acetylsalicylate and D-lactate and some electrical properties were studied in isolated frog gastric mucosae. With both compounds, the unionized acids permeated much more rapidly than the ionized conjugate bases. The permeability coefficient for unionized acetylsalicylic acid increased from 0.27 to 0.43 μmoles, h^{-1}, cm^{-2}, mM^{-1} when its luminal concentration was increased above 3 mM. Simultaneously there was an increase in mucosal ion conductance. Acetylsalicylic acid has been shown previously to increase gastric mucosal permeation of ions and uncharged larger molecules. The present results indicate that above a threshold concentration the unionized form also enhances absorption of this drug per se. Unionized D-lactic acid had no effect of mucosal ion conductance and the permeability coefficient (0.07 μmoles, h^{-1}, cm^{-2}, mM^{-1}) was independent of its luminal concentration.

INTRODUCTION

It is generally accepted that unionized weak acids are more rapidly absorbed from the stomach than the ionized conjugate bases. Assuming low tissue or blood levels of the compound, the rate of absorption is considered proportional to its concentration in the gastric contents (16, 17, 19).

Acetylsalicylic and some other acids, however, if present in the unionized form in stomachs with a low intraluminal pH increase the mucosal permeability to ions and uncharged larger molecules.The ionized conjugate bases have much smaller or no effects. This has been observed in several species in vivo (1, 5, 7, 8, 9) and in vitro (2, 6, 11, 18) and it therefore seemed of interest to examine whether the permeability increasing effect of unionized acetylsalicylic acid could also affect gastric mucosal permeation of this drug per se. For comparison, experiments were also performed with D-lactic acid. This acid is devoid of permeability effects in the stomach (10) and is not metabolized by vertebrate tissues. Transmucosal migration of the acids was studied in vitro, which

permitted simultaneous determination of some electrical characteristics of the mucosa.

MATERIALS AND METHODS

Frogs (Rana temporaria) obtained from Firma Panzer, Lauingen-Donau, GFR, were kept in tap water at 8 - 10°C for up to two months before use. They were force-fed 100 - 200 mg of liver once a week. After section of the spinal cord, the abdomen was opened and the stomach removed. The fundic mucosa was separated from the rest of the stomach wall by blunt dissection and mounted as a membrane between the two compartments of a perspex chamber (2). The exposed mucosal area was 1.8 cm^2.

On each side of the mucosa there was 20 ml of solution which under standard conditions contained Na^+ 102.4, K^+ 4.0, Ca^{2+} 1.8, Mg^{2+} 0.8 and Cl^- 91.4 mM (3). The unbuffered luminal (secretory) side was gassed with 100 % O_2. The pH on this side was kept constant at a preselected level by continuous titration with 10 mM NaOH under automatic control from a pH-stat equipment (Radiometer, Copenhagen, Denmark). The nutrient (submucosal) solution (pH 7.20) was buffered with HCO_3^- (17.8 mM) and phosphate (0.8 mM) and gassed with 5 % CO_2 and 95% O_2 (vol/vol). All experiments were performed with the mucosa in the short-circuited state, the transepithelial electric potential difference (PD) being brought to zero by current from an external source applied to the mucosa through two Ag/AgCl electrodes. The magnitude of the current was measured from the voltage drop across a precision resistor. The open circuit PD was measured every 10 min via two matched calomel electrodes connected to a high-input impedance voltmeter. The electrical resistance (ionic conductance) of the mucosa was determined from the immediate change in open circuit PD when a fixed current (30 μA, cm^{-2}) was sent through the mucosa in either direction. All experiments were performed at 20°C.

When used, acetylsalicylate or D-lactate was added to the luminal solution (replacing Cl^-) to concentrations between 1 and 10 mM. Transepithelial flux of these compounds was measured by adding carboxy ^{14}C-acetylsalicylate or uniformly labelled ^{14}C-D-lactate (0.2 μCi, ml^{-1})to the solutions. Samples (0.5 ml) were drawn from the nutrient (trans) side every 30 min and ^{14}C activity was determined by liquid scintillation counting (Beckman CPM 250). At least 4,000 counts above background were counted. To avoid introduction of transepithelial hydrostatic pressure differences, 0.5 ml of fluid was redrawn every 30 min from the luminal side also.

Two types of experiments were performed. In a first series the luminal to nutrient side transport of the unionized acids was studied. After a 60-min control period (luminal pH 7.12) the standard solution was replaced by solutions containing acetylsalicylate or D-lactate at pH 3.00. Dilution by remnants of

standard solution was determined by samples taken from the luminal side 3 min after the change. If necessary, luminal pH was returned to 3.00 by the addition of small amounts of isotonic HCl. The transmucosal migration of acetylsalicylate or D-lactate was then studied for 8 consecutive 30 min periods. At pH 3.00, 89 % of D-lactate (pK_a 3.90) and 76% of acetylsalicylate (pK_a 3.50) is in the unionized form.

In a second series the transmucosal migration of the conjugate base was examined. After a 60-min control period H^+ secretory arrest was induced by the addition of SCN^- (20 mM, replacing Cl^-) to both sides. This was necessary to avoid formation of unionized acid by secreted H^+, and only mucosae showing absence of any measurable H^+ secretion within 60 min (10 out of 18) were used for further experiments. The luminal solution was then changed to a solution containing 5 mM acetylsalicylate or D-lactate at pH 7.12. SCN^- was present throughout the experiments.

Carboxy-^{14}C-acetylsalicylate and uniformly labelled ^{14}C-D-lactate were purchased from the Radiochemical Centre, Amersham, England. D-lactate (containing 95% D- and 5% L-lactate) and acetylsalicylic acid were obtained from Sigma Chem. Co., St. Louis, Mo., USA. All chemicals were of highest available purity.

RESULTS

Transport of the unionized acids. Transmucosal migration of both acetylsalicylic and D-lactic acid attained steady state rates 30 - 60 min after the start of the luminal side exposure. With D-lactic acid there was always a linear relation between luminal concentration and rate of transmucosal migration (Fig. 1). With 5 and 10 mM acetylsalicylic acid, the transport was considerably greater than was expected from the linear relation obtained with lower (1 - 3 mM) concentrations. The permeability for acetylsalicylic acid thus increased at high luminal concentrations of this drug.

The effects of luminal acetylsalicylic acid on the electrical properties of the mucosa are shown in Table 1. All concentrations (1 - 10 mM) decreased ($p < 0.01$ for all) the transepithelial electric potential difference (PD) and the short-circuit current. The effects were greater with higher concentrations. The electrical resistance was unaffected by the acid in a concentration of 1 or 2 mM and increased ($0.02<p< 0.05$) somewhat with 3 mM. With 5 or 10 mM acetylsalicylic acid there was a significant ($p<0.01$ for both) decrease of this parameter, indicating an increase in mucosal ion conductivity permeability.

No changes in electrical properties were observed with luminal D-lactic acid.

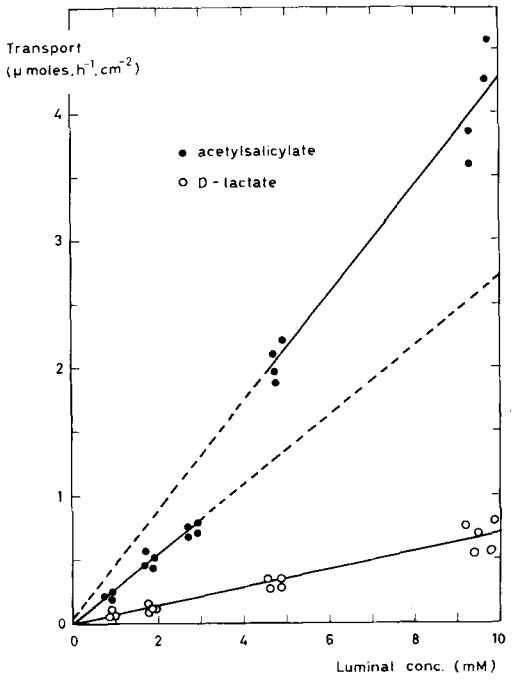

Fig. 1. Transmucosal migration of unionized (luminal pH 3.00) acetylsalicylate and D-lactate at luminal concentrations between 1 and 10 mM. Each point is the steady state rate 60-90 min after the start of exposure in one experiment. Lines were calculated by the method of least squares. Note that acetylsalicylate has a higher permeability at luminal concentrations above 3 mM.

Transport of ionized acetylsalicylate and D-lactate. These mucosae were pretreated with 20 mM SCN^- to abolish H^+ secretion. SCN^- *per se* increased ($0.01 < p < 0.02$) the electrical resistance from 419 ± 37 to 478 ± 27 ohm·cm^2 (means $\pm$ SE, n = 10) but did not affect the PD or short-circuit current.

Steady state rates of transmucosal migration of acetylsalicylate or D-lactate (5 mM, pH 7.12) were obtained during the period 60 - 90 min after the luminal side application. Permeation of the anions was much smaller than for the unionized acids (Table 2). Luminal application of the anions did not affect the electrical parameters of the mucosae.

DISCUSSION

Acetylsalicylic acid increases the permeability of the gastric mucosa to ions and larger molecules if present in the unionized form in the gastric lumen. While the frog gastric mucosa *in vitro* is normally not permeable even to the trisaccharide raffinose (mol. wt. 504 daltons), dextran molecules with a maximal mol. wt. of about 30,000 daltons permeated the mucosa after exposure of the luminal side to unionized acetylsalicylic acid (6). On intragastric instillation of 15 - 20 mM, the acid also greatly increased the normally very small gastric absorption of inulin (weight average mol. wt. 5,400 daltons) from the cat stomach (7) and resulted in the appearance of serum albumin in the dog stomach pouch (1). Studies in the guinea pig (8) indicate that unionized acetylsalicylic acid induces absorption of dextran molecules with a maximal mol. wt. of about 25,000 daltons, but only at concentrations above a threshold

Table 1. Effects of unionized acetylsalicylic acid (ASA) on electrical properties.

	PD (mV)	I_{sc} (μmoles, h^{-1}, cm^{-2})	Resistance (ohm · cm^2)
Control	32.1±4.4	2.59±0.21	370±39
1 mM ASA	25.2±2.4	2.25±0.08	359±48
Control	20.7±2.5	1.62±0.09	449±48
2mM ASA	16.7±2.8	1.36±0.06	429±70
Control	27.6±2.6	2.49±0.18	367±28
3 mM ASA	13.9±1.8	1.20±0.33	420±42
Control	24.3±2.8	2.28±0.25	366±20
5 mM ASA	1.6±0.4	0.30±0.04	122±23
Control	22.6±2.4	2.01±0.21	360±23
10 mM ASA	3.6±2.3	0.60±0.42	130±42

After a 60 min control the luminal surface of the gastric mucosa was exposed to unionized acetylsalicylic acid (pH 3.00). Mean values ±SE of electric potential difference (PD), short-circuit current (I_{sc}) and electrical resistance 60 - 90 min after the start of the exposure are presented. n = 4 for all concentrations.

Table 2. Transmucosal migration of acetylsalicylate (ASA) and D-Lactate

Compound	Unionized	Ionized
ASA (1-3mM)	0.272±0.060 (n=11)	0.028±0.008 (n=5)
ASA (5-10mM)	0.428±0.065 (n=8)	-
D-lactate	0.073±0.028 (n=16)	0.005±0.001 (n=5)

Steady state values of the permeability coefficients (μmoles, h^{-1}, cm^{-2}, mM^{-1}), 60 - 90 min after luminal instillation of the compounds are presented. Values for the unionized acids were obtained with luminal pH at 3.00. The coefficients $\pm S_{x.y}$ were calculated by the method of least squares (cf. Fig. 1). Means ± SE of permeability coefficients for the ionized forms were determined after instillation of 5 mM of the compounds at pH 7.12.

level (5 - 10 mM). Acetic and some other fatty acids (100 - 170 mM) have permeability-increasing effects similar to those of acetylsalicylic acid (1, 2, 5) while very high concentrations (170 - 700 mM) of D- and L-lactic acid were without effect on ion permeability in the cat stomach (10).

It was found in the present study that unionized acetylsalicylic acid at luminal concentrations above 3 mM permeated the mucosa considerably more rapidly than at lower concentrations. The higher permeability was associated with a decrease in mucosal electrical resistance (increase in ion permeability). The results indicate that intragastric acetylsalicylic acid, in addition to affecting the permeability for ions and macromolecules, also promotes absorption of the drug per se.

This may also occur in vivo, since acetylsalicylate effects on ion permeability (1, 6, 9), migration of macromolecules (6, 8) and mucosal electrical properties (6, 14, 18) are very similar in vivo and in vitro. Most previous studies on gastric absorption of acetylsalicylic acid have employed either very low (16, 17) or very high (1, 9, 12) concentrations of the acid.

The mechanism of the permeability-increasing effects of some unionized weak acids and the absence of effects of the conjugate bases is not fully clarified. It is likely, however, that the unionized acid rapidly permeates into mucosal cells, while efflux of the anion (formed at the higher intracellular pH) is slower and experimental evidence for mucosal intracellular accumulation of the acids has been presented (2, 12). The much more rapid migration of the unionized acids than the anions (Table 2) supports this concept. It seems probable that compounds with effects on gastric mucosal cell metabolism, as described for acetylsalicylate (15, 18) thereby attain intracellular concentrations sufficiently high to affect various aspects of tissue function, including maintenance of normal permeability characteristics. Unionized acetylsalicylate in a concentration as low as 1 mM decreased the mucosal electric potential difference and short-circuit current (Table 1). This is in agreement with previous observations (18) that the drug, by effects on cell metabolism, inhibits electrogenic transport of H^+ and Cl^- in the gastric mucosa. The absence of effects of D-lactate on the permeability and electrical properties may reflect both its considerably slower migration (Fig. 1) and the less deleterious effects of this compound on tissue metabolism.

Recent studies indicate that the surface epithelial cells of the gastric mucosa transport HCO_3^- into the gastric lumen and that this transport protects the mucosa from damaging effects of intraluminal HCl by alkalinization of the immediate vicinity of the luminal cell membranes (3, 4, 13). Acetylsalicylate is a potent inhibitor of this transport (11, 12) and it is possible that the findings of a threshold concentration for an effect of acetylsalicylic acid in vitro (Fig. 1) and in vivo (8) reflect mucosal secretion of HCO_3^- sufficient to convert

only limited amounts of unionized acid into the less permeable conjugate base.

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