Permselective Characteristics of Rabbit Buccal Mucosa

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INTRODUCTION

Hydrophobic solutes are thought to diffuse readily through hydrophobic membranes and hydrophilic solutes through hydrophilic membranes. The delivery of ionic drugs through biological membranes is limited by their weak partitioning and low diffusivity. If the buccal epithelium is treated as a nonselective membrane, the relative fluxes of various ions would depend not only on their relative transport numbers in free solution but also on the net charge of the buccal epithelium. The ionizability of buccal tissue renders the buccal epithelium selective to ion transport, a property termed permselectivity. To exploit the buccal route for delivery of charged compounds such as peptides and proteins, the permselective properties of the membrane need to be studied. Permselective properties of the cornea have been well characterized (1). The objective of this work was to investigate the presence and origin of fixed charge on the buccal epithelium and characterize its permselective properties.

MATERIALS AND METHODS

Tissue Collection

Male albino New Zealand rabbits (Bakkom's Rabbitry, Viroqua, WI) were sacrificed by an intravenous injection of Na-pentobarbital into a marginal ear vein. The buccal cavity was laid open after two incisions on each side of the head, and the mucosa, muscle, and underlying submucosal tissue was cut away and separated from the connective tissue. The epithelium was then separated and cleaned from the remaining muscle. The tissue was mounted on the diffusion cell within 10 to 15 min after the animal's death. Earlier studies in this laboratory found the tissue to be viable postexcision for up to 4 hr (2). The diffusion cells were a modification of cells described in a previous study (3). This cell has been characterized with respect to its hydrodynamics (4). The cells were maintained at 37 ± 0.5°C and bubbled with a 95%O_2/5%CO_2 mixture to maintain tissue integrity.

Chemicals and Solutions

Unless otherwise stated, all chemicals were purchased from Sigma Chemical Co. (St. Louis, MO) and were used as received. All solutions were prepared using distilled deionized water. All electrokinetic measurements were performed using KCl, glucose, and sucrose at different concentrations.

Potential Measurements

Diffusion potential measurements were performed using Ag/AgCl electrodes as described previously (1). Briefly the epithelial side was bathed with 160 m*M* KCl solution in the donor chamber, while the serosal side was bathed with 16 m*M* KCl solution in the receiver chamber of an *in vitro* diffusion cell. The solutions on either side were rendered isoosmotic using sucrose and adjusted to 290–310 mOsm by a Wescor 55D vapor pressure osmometer (Logan, UT). The pH's of the solution on either side were identical at all times. pH was varied from 2.0 to 10.0 with the addition of HCl or KOH as needed. The steady-state potential achieved within 20 min was measured. The pH's of the solutions were measured immediately after potential measurements.

The membrane potentials were determined from the observed steady-stage potential after correction for electrode potential as shown by Eq. (1).

$$\Delta E_m = \Delta E_{obs} - \Delta E_{electrode} \quad (1)$$

where

$$\begin{aligned} \Delta E_m &= \text{membrane potential difference} \\ \Delta E_{obs} &= \text{observed potential difference} \\ \Delta E_{electrode} &= \text{electrode potential difference} \\ \Delta E_{electrode} &= -RT/F \ln (a_2^{KCl}/a_1^{KCl}) \quad (2) \end{aligned}$$

where R is the gas constant, T is the absolute temperature, F is Faraday's gas constant, and a_2 and a_1 are the activities of KCl on the donor and receptor side, respectively.

Streaming potential in the buccal epithelium was measured using an osmotic pressure gradient. The buccal epithelium was bathed on either side with solutions of a mixture of KCl (146 m*M*) and glucose (28 m*M*). The osmotic pressure was generated by the addition of 100 m*M* sucrose on the epithelial side. The osmolarity of the serosal solution was adjusted to 290–310 mOsm and the pH of the solution varied from 2 to 10 as described earlier. The steady-state potentials were measured at each pH.

Resistance measurements were performed as described previously (1). Briefly, the excised buccal membrane was placed in a side-by-side diffusion cell with both compartments filled with an identical KCl solution or HEPES buffer. The connection of the four electrodes is as shown in Fig. 1. The resistance at each time point was calculated from the slope of the line obtained by plotting potential difference vs current, applying Ohm's law, and after correcting for the resistance in absence of the membrane.

RESULTS AND DISCUSSION

Effect of pH on Diffusion Potential

The diffusion potential is defined as the potential differ-

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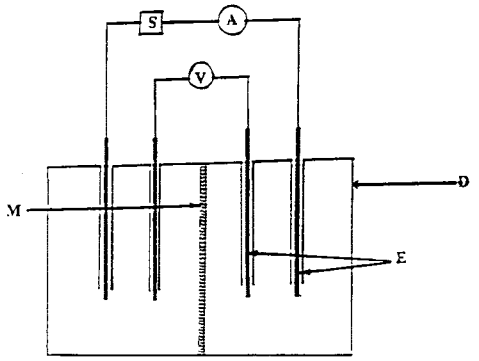

Fig. 1. Circuit diagram for measurement of electrical resistance. E, Ag/AgCl electrodes; S, current source; M, membrane; D, diffusion cell; A, ammeter; V, voltmeter.

ence produced across a charged membrane by the movement of ions under the influence of a concentration gradient(s). The potential changes as a function of time and reaches a steady-state value within 15 to 20 min. When the membrane was bathed on either side with an identical isoosmotic solution of KCl (bicarbonate free), no potential difference was observed (0 ± 0.5 mV), because an ion pump may not show significant potential under these experimental conditions. Therefore, potential differences arising from ionic concentration and osmotic gradients were interpreted wholly as either diffusion or streaming potential, respectively. Figure 2 illustrates the effect of pH on steady-state diffusion potential.

The results show that below pH 3.0 the magitude of the diffusion potential varies with pH, and at pH values below 2.7, the sign of the potential is actually reversed. At pH 2.7, the potential is zero, which is defined as the isoelectric point, p*I*. At pH 2.7 ± 0.25 (n = 5), the membrane does not show any preference to transport of either ion, suggesting that it is electrically neutral. This result is consistent with the concept of a membrane isoelectric point at approximately 2.7. Since the potential difference is positive from pH 2.7 to pH 10, the mucosal side is positive with respect to the serosal side at physiological pH. Below the isoelectric point the membrane changes its polarity and possesses a net positive charge.

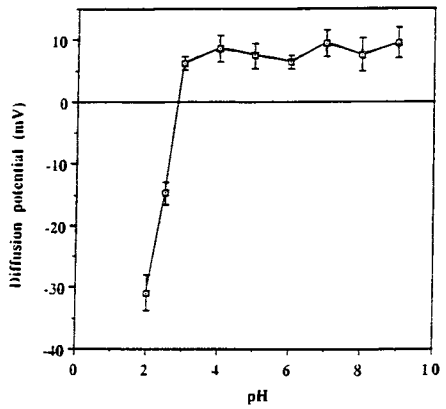

Fig. 2. Effect of pH on KCl diffusion potential in rabbit buccal mucosa. Note that the reversal of sign of potential occurs at the isoelectric point of 2.7. The signs of potential are reversed for ease of presentation. Error bars represent SE; n = 5.

Membrane charges may result from dissociation of the basic and acidic residues in proteins forming membrane pores and, therefore, could regulate ion movement across the cell and provide cell wall adhesion. The charges may be located along pores and channels or very near the cell surface itself. The isoelectric points for most biological membranes range from 2.7 to 5.3. The small intestine has an isoelectric point of 2.7 (5), the skin has a value of 5.3 (6), and the cornea 3.1 (1). It is speculated that at physiological pH the negative fixed charge of the buccal membrane results from the greater number of ionizable carboxylic acid groups than amino groups of the proteins lining the transport pathway. Other contributing groups include sialic acids, sulfates in proteoglycans, and phosphates.

The intercellular space of the buccal membrane at the superficial layer contains an abundant quantity of products discharged from membrane coating granules (MCG) (7). One of the components of MCGs is thought to be glycoproteins, composed chiefly of proteoglycans formed by linkage between glycosaminoglycans and proteins. The titration parameters of carboxyl groups of proteoglycan are shown in Table I. The fixed charges on the buccal epithelium could result from the carboxyl groups of the various proteoglycans. Hyaluronic acid and chondroitin sulfate have been reported to have pK_a's of 2.98 and 2.90, respectively (8).

We may write the ionization of the acidic site in the form

$$AH \rightleftharpoons A^- + H^+$$

and that of the basic site as

$$BH^+ \rightleftharpoons B + H^+$$

At a pH near neutrality the sites would be in the form A^- and B so that the membrane would bear a net negative charge, be relatively impermeable to anions, and function as an ion-exchange membrane for cations. At acidic pH below the isoelectric point both sites become protonated to form AH and BH^+ so that the membrane would bear a net positive charge, be relatively permeable to cations, and function as an anion exchanger. Hence the membrane behaves as an ion-exchange resin, being cation selective at higher pH's above p*I* and anion selective at lower pH's below p*I*. This membrane property is important in effectively delivering charged species. Besides avoiding first-pass metabolism, one advantage of buccal drug delivery is that one can modify the membrane pH locally in order to favor the permeation of particular charged species.

The similarity between Fig. 2 and the titration curve of an amphoteric substance suggests that the ionic permeation pathway of the cell membrane of buccal epithelium contains

Table I. pK_a of Acid Groups of Glycosaminoglycans[a]

Compound	pK_a
Hyaluronic acid	2.98
Chondroitin	2.90
Desulfated chondroitin sulfate	3.10
Dermatan sulfate	3.63
Desulfated dermatan sulfate	3.30

[a] Source: Ref. 8.

both acidic and basic groups. The membrane charges are responsible for the observed differences in the permeability between cations and anions. That the effect of low pH on permeability is due to protonation of membrane components rather than protein denaturation or other effects was suggested by the complete reversibility of permeability changes in the buccal epithelium after brief exposure to low pH.

Effect of pH on Streaming Potential

An electrokinetic phenomenon, the streaming potential, was studied in order to confirm the results obtained from diffusion potential studies. Streaming potential is defined as the potential difference produced by flow of ions across the charged membrane under the influence of either a hydrostatic or an osmotic pressure gradient. If the membrane bears a negative fixed charge, the aqueous channels through the membrane will contain more mobile cations than anions. The water flow resulting from either a hydrostatic or an osmotic gradient thus sweeps out fluid with an excess of cations resulting in electrical potential. Preliminary work with buccal epithelium demonstrated that streaming potential measurements on osmotic gradients were unstable in the absence of glucose (Fig. 3a). In the presence of glucose the potentials were quite stable, (Fig. 3b) and hence the steady-state potential value could be assigned at each pH. Stability of the potential is related to the energy available from hexose metabolism (7). Unlike studies in the cornea (1), where streaming potentials were generated using hydrostatic pressure, in the present study they were generated using osmotic pressure by the addition of sucrose on one side. The effect of pH on streaming potential (Fig. 4) indicates that the membrane has an isoelectric point of 2.6 and is cation selective above pH 2.6, while below pH 2.6 it reverses its polarity and is anion selective. These results closely agree with those obtained from diffusion potential studies.

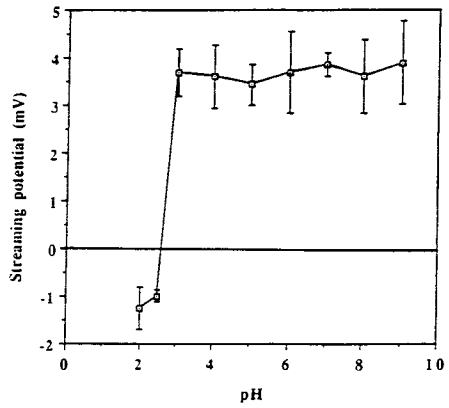

Fig. 4. Effect of pH on streaming potential in rabbit buccal mucosa. Note that the reversal of sign of potential occurs at the isoelectric point of 2.6. Error bars represent SE; $n = 5$.

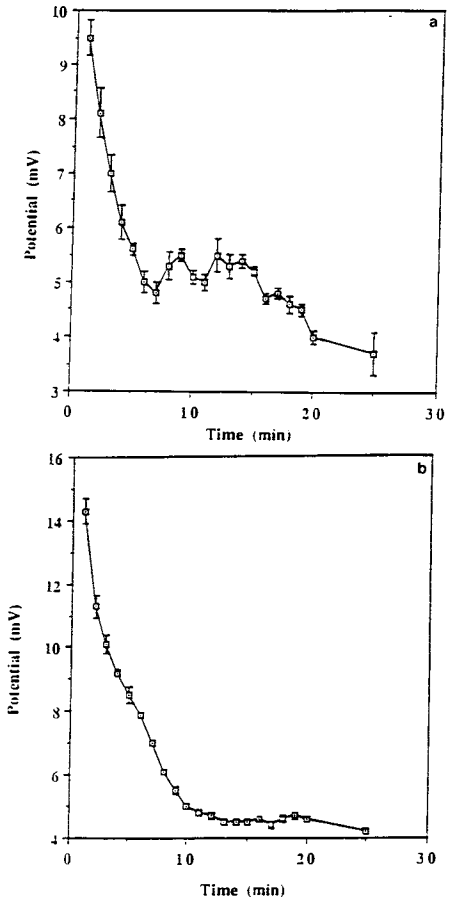

Fig. 3a. Streaming potentials generated by an osmotic pressure gradient in the absence of glucose at pH 7.0 in rabbit buccal mucosa. Note the instability of potentials. Error bars represent SE; $n = 3$.
Fig. 3b. Streaming potentials generated by an osmotic pressure gradient in the presence of glucose at pH 7.0 in rabbit buccal mucosa. Error bars represent SE; $n = 3$.

Electrical Resistance

Figure 5 shows the resistance of buccal epithelium as a function of time in Hepes buffer solution at pH 7.4. The resistance increases with time and then reaches a steady-state value of 682 Ω cm^2, and therefore this epithelium can be classified as tight. Table II lists the resistances of several different membranes reported in the literature, showing that the resistance of buccal epithelium is less than that of skin. The absence of keratin and tight junctions in buccal epithe-

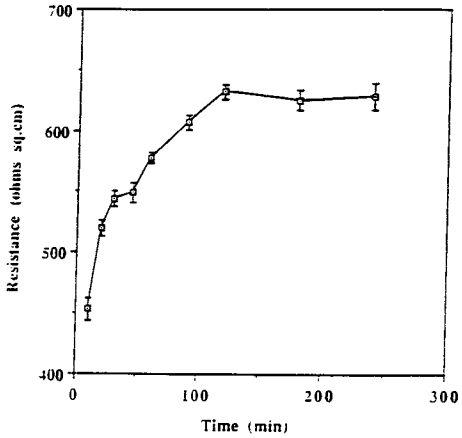

Fig. 5. Profile showing electrical resistance of rabbit buccal epithelium in HEPES buffer solution at pH 7.4. Error bars represent SE; $n = 4$.

Table II. Electrical Resistances of Various Epithelia

Tissue	Species	Resistance (Ω cm^2)	Ref. No.
Gastric mucosa			
Antrum	Necturus	1730	9
Fundus	Necturus	2230	9
Small intestine			
Duodenum	Rat	98	9
Jejunum	Rat	51	9
Ileum	Rabbit	100	9
Colon	Rabbit	385	9
Cornea	Rabbit	510	1
Amphibian skin	Toad	763	9
	Frog	8700	9

lium might be responsible for such a low value. However, the resistance is higher than that of intestine. Intestinal tissue, unlike buccal tissue, does not show the presence of a superficial layer (dead cells).

The resistance of the cornea is lower than that of the buccal epithelium, probably because of the greater thickness of the buccal membrane compared to the cornea. The isoelectric points of the two are significantly different because of the nature of the fixed charged groups at the surface. While the surface charges in the cornea are attributed primarily to acidic and basic groups of stromal collagen and sulfate groups of proteoglycans (7), the two major constituents of solid cornea matter, the surface charges in buccal epithelium may be due primarily to glycosaminoglycans, *viz.*, hyaluronic acid and chondroitin sulfate.

CONCLUSIONS

Electrophysiology studies show that the isoelectric point of the buccal epithelium is 2.7. At physiological pH the membrane is cation selective, whereas below the isoelectric point, the membrane reverses its polarity and is anion selective. At the isoelectric point the membrane is nondiscriminating to either ion. The magnitude of resistance is less than that of a tight tissue such as skin. The present study indicates that in order effectively to deliver charged compounds across buccal epithelia, one can control the degree of protonation and electrostatic shielding of the charged groups on the membrane and create favorable conditions for their transport.

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