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Transport mechanisms of the cornea: characterization of barrier permselectivity

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Summary

Corneal permselectivity was investigated in vitro by measurement of membrane electrokinetic potentials generated either by ionic concentration gradient (diffusion potential) or hydrostatic pressure gradient (streaming potential). Studies on the effect of pH on these potentials indicates dual-selective character to passage of ions across the cornea. The magnitude and polarity of the selectivity are controlled by the degree of protonation of ionizable sites within the cornea. The cornea presents an isoelectric point (p*I*) of 3.2. At physiological pH and pH above p*I*, the cornea behaves as if it is negatively charged and allows preferential passage of positive ions with respect to negative ions. Below p*I*, the reverse is valid. Lowering the ionic strength of the bathing solutions results in an increase in membrane ionic discrimination as well as membrane electrical resistance. Tissue viability and integrity, the two important membrane properties involved in the overall transport process, were investigated. The mechanisms and the significance of membrane permselectivity to epithelial drug transport are discussed.

Introduction

Transport across epithelial membranes has long been recognized not only for its physiological importance but also for its dominant role in non-parenteral drug delivery. Different transport mechanisms such as simple passive diffusion active and other specialized transport processes have been defined in varying detail. However, less attention has been paid to other important aspects of epithelial transport, i.e. the ability of the epithelia to discriminate or show preference to transport of molecules of different charges, the so-called permselective property of the membrane. The present work is concerned with the electrical properties and the permselectivity characteristic of the cornea. The experiments were designed to test the hypothesis of the existence of fixed charged groups in the cornea and the conditions by which their polarity and density can be regulated. The first experiments describe the potential difference in the presence of concentration gradients or diffusion potentials; the second concerns the effect of ionic strength on these potentials and the resis-

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tance of the cornea; thirdly the streaming potentials resulting from hydrostatic pressure gradients; and finally tissue viability and integrity. As regards transport mechanisms, understanding mechanisms of membrane permselectivity would allow a more effective way of delivering drugs, especially those with charges. In other cases, it may be possible to transiently modify membrane charges to increase transport of specific compounds. The knowledge from this study would also be applicable to transport in other epithelia.

Barrier permselectivity is a complex phenomenon which combines not only passive contribution from electrostatic shunt activity, probably due to membrane-fixed charges, but also active contribution from cell membrane activity. The latter is generally a reflection of carriers and pumps residing in the membrane. Since they are all interrelated, i.e. active potential can enhance or retard passive permeation depending on polarity of the potential, the interpretation of permselectivity data is normally difficult unless the two contributions can be separated. In the cornea, more is known about active permselectivity due to its importance in conservation of transparency and maintenance of its metabolic function. Accumulated evidence indicates that this process is only specific to certain ions and varies from species to species (Zadunaisky, 1978). In rabbit cornea, where the majority of data were obtained, active contribution was found to come from an inward sodium transport from tear to the aqueous humor side (Donn et al., 1959; Green, 1965) and an outward chloride transport in the reverse direction (Zadunaisky, 1966; Klyce et al., 1973). In the present paper, the focus is on the lesser-known passive permselectivity of the cornea. The mechanisms and some factors governing this process are examined.

Experimental

Materials and methods

Animals. Male albino New Zealand rabbits (New Franken, WI) weighing between 2.5 and 3.0 kg were used throughout the studies. Lighting was maintained on a 24-h basis in the caging facility, and the animals were fed a regular diet with no restriction on the amount of food or water consumed.

In vitro diffusion studies. Rabbits were sacrificed with an intravenous injection of an overdose of sodium pentobarbital given via the marginal vein of an ear. The whole eye was enucleated from its socket with care to avoid any physical contact or contamination of the corneal surface. The mounting procedure was according to that described by Schoenwald and Huang (1983) with slight modification. A suction device with inner corneal ring attached was placed on the cornea. The suction was gently operated to maintain corneal curvature during the mounting procedure. Blood clots were removed and the lateral connective tissue was scraped over the suction device. Eyeball muscle was removed and the remaining tissue was tied to the ring with a surgical thread. After removing the posterior half of the sclera, the remaining half was scraped over the ring, and the lens, vitrous, retina, and iris/ciliary body were subsequently removed. Finally, the outer corneal ring was placed over the tissue-attached inner ring and whole ring set was removed from the suction device, and mounted in the diffusion cells (Medical Instruments Department, University of Iowa Medical Center, IA). Silicone grease was applied to the contact surfaces to prevent possible leakage of the bathing fluid. The whole mounting process was normally completed within 10 min after animal's death. Immediately after the tissue was mounted, the chambers were filled with bathing solution. Bubbling of O_2/CO_2 (95/5%) was initiated and the solutions were constantly stirred with a propeller driven by an electrical motor. Stirring was close to the surfaces of each side of the membrane to minimize liquid junction potentials at the boundary layers. All experiments were carried out at 37 °C using a constant temperature bath (American Scientific Products, McGaw Park, IL) with external circulator connecting through water jacket of the diffusion cells. A 1.04 cm^2 area of tissue was exposed to the donor and receptor compartments, each having the volume of 7 ml.

Solution preparation. Unless otherwise stated, all chemicals were used as received and all solutions were made using deionized distilled water. In

most experiments relating to KCl diffusion potential and streaming potential measurements, isotonic KCl solutions containing 160 mM KCl were used. In some experiments, where the effect of ionic concentration gradient and ionic strength of the solutions were studied, an iso-osmotic amount of a non-electrolyte, sucrose, was added. The final osmolarity of all test solutions was maintained at 290–295 mOsm as determined by a Wescor 5500 vapor pressure osmometer (Logan, UT). Detailed compositions of the test solutions are indicated in each result section. In the viability and tissue integrity experiments, glutathione-bicarbonated Ringer's solution (GBR), according to O'Brien and Edelhauser (1977) was used. This solution is reported to preserve integrity of the excised cornea for up to 6 h. The solution was prepared in two parts; the first part contained sodium chloride (12.4 g/l), potassium chloride (0.716 g/l), monobasic sodium phosphate monohydrate (0.206 g/l), and sodium bicarbonate (4.908 g/l); the second part contained calcium chloride dihydrate (0.230 g/l), magnesium chloride hexahydrate (0.318 g/l), glucose (1.80 g/l), and oxidized glutathione (0.184 g/l). Equal parts of both solutions were mixed prior to use. Solutions were stored in the refrigerator and used within 3 weeks.

Electrical measurements

Electrical potential difference. To measure the potential difference across the cornea, a high input-impedance, microvolt multimeter (Keithley Model 197, Keithley Instruments) was used. Ag–AgCl electrodes were used as sensing devices. They were prepared from silver wires (99.9% purity, Aldrich) of 5 cm × 1 mm size. They were cleaned by surface abrasion with a fine sanding paper, placed in concentrated HCl for 10 min, and thoroughly washed in distilled water. The electrodes were then chlorided with direct current of about 0.1 mA for 6 h in 0.5 M KCl. The finished electrodes were kept short-circuited, in pairs, in 0.16 M KCl. They were stored in the dark (AgCl is photosensitive) and allowed at least 24 h for intraelectrode potential stabilization prior to use. Asymmetry between electrodes was tested prior to each potential measurement. Only pairs of electrodes differing in potential by less than 0.1 mV in 0.16 M KCl were used.

Electrical resistance. Corneal resistance was measured by applying current pulses and recording the potential drop across the membrane. Two pairs of Ag/AgCl electrodes were used for this measurement; the first pair (located 1 cm from each side of the cornea) recorded potential difference and the second pair (located 2 cm from each side) was used to inject current pulses. In all experiments, the anode was placed in the epithelial (donor) solution and the cathode was placed in the endothelial (receptor) solution. Pulses of variable duration (1–10 s) and intensity (up to $\pm 50\ \mu A \cdot cm^{-2}$) were tapped off potentiometrically from a current source and measured by the Keithley multimeter. All resistances were calculated from the slope of the current and potential difference plots. To correct for the potential drop due to solution resistance between the sensing electrodes and the membrane, measurements were carried out before each membrane resistance determination using the same bathing solution but without the membrane in the diffusion cell. Membrane resistance was then calculated by subtracting from the resistance obtained with the membrane the resistance obtained without the membrane.

Theory and Calculations

The transmembrane potential difference (ΔE_m) was obtained from the difference in the observed potential difference (ΔE_{obs}) and the electrode potential difference (ΔE_e) calculated from solution ionic activities according to the Planck and Henderson equation (see MacInnes, 1961, p. 231–233).

$$\Delta E_m = \Delta E_{obs} - \Delta E_e \tag{1}$$

$$\Delta E_e = -(RT/F)\ \ln\left(a_2^{Cl}/a_1^{Cl}\right) \tag{2}$$

$$a^{Cl} = a^{KCl}* \tag{3}$$

$$a^{KCl} = g_{\pm} \cdot m \tag{4}$$

* Assumption according to MacInnes (1961).

where a^{Cl} = chloride activity; a^{KCl} = KCl mean ionic activity; $g_{\pm}$ = KCl mean ionic activity coefficient obtained from Robinson and Stokes (1959); m = KCl molality; subscripts 1 and 2 refer to the two bathing solutions; R = gas constant; and F = Faraday constant. The effect of HCl and KOH, used to adjust the pH of the solutions, on KCl activities was negligible in the range of concentrations used in the experiments (typically ≤ 0.01 fraction of the total ionic mass). The result was supported by previous work conducted by Harned and Gancy (1958) and Harned and Gancy (1937).

Consider how the diffusion of different ions gives rise to electrical potential differences. The analysis is based on the Nernst-Planck flux equation of monovalent KCl solution (Nernst, 1888; Planck, 1890):

$$J^K = -u^K RTC^K/a^K(\partial a^K/\partial x) - u^K C^K F(\partial E/\partial x) \quad (5)$$

$$J^{Cl} = -u^{Cl} RTC^{Cl}/a^{Cl}(\partial a^{Cl}/\partial x) + u^{Cl} C^{Cl} F(\partial E/\partial x) \quad (6)$$

where J, u, C, a, x, and E represent flux, mobility, concentration, activity, distance, and electrical potential respectively. Since bulk electroneutrality of each solution must be preserved, it follows that $C^K = C^{Cl}$, $a^K = a^{Cl} = a^{KCl}$, and $J^K = J^{Cl}$. Equating Eqns. 5 and 6 and rearranging terms yields:

$$\partial E/\partial x = -RT/F(u^K - u^{Cl}) /(u^K + u^{Cl})\partial(\ln a^{KCl})/\partial x \quad (7)$$

and integrating across the thickness of the membrane:

$$\Delta E = E_2 - E_1 = -RT/F(u^K - u^{Cl}) /(u^K + u^{Cl}) \ln(a_2^{KCl}/a_1^{KCl}) \quad (8)$$

Defining the transport number (t) of a species as the fraction of charge carried by it (for monovalent solution, $t^K = u^K/(u^K + u^{Cl})$ and $t^{Cl} = u^{Cl}/(u^K + u^{Cl})$, Eqn. 8 can be rewritten as:

$$\Delta E = -RT/F(2t^K - 1) \ln(a_2^{KCl}/a_1^{KCl}) \quad (9)$$

Eqn. 9 provides the important expression relating transmembrane potential difference, activity gradient, and potassium transport number. With this equation, information on the type and degree of ionic discrimination presented by the membrane (permselectivity) can be obtained from knowledge of the sign and magnitude of membrane potentials. It should be noted that this expression is derived without any limiting assumptions concerning membrane composition or structure, and electrical field within the membrane. A similar expression was also derived, using irreversible thermodynamics, by Stavermann (1952) and Gunn and Curran (1971).

Results and Discussion

The use of KCl and problems associated with active potential

In this study, KCl was used because K^+ and Cl^- have similar mobilities and hydrated radii in free solution (Robinson and Stokes, 1959). Thus, the problem of liquid junction potential was minimized and membrane permselectivity to solutes of different charges can then be interpreted directly without the effect of solute size. Another problem in interpreting diffusion and streaming potential data, as mentioned earlier, is the presence of active potential across the cornea. This potential was shown to be in the order of 25 mV (Maurice, 1967; Klyce, 1972) and was accounted for mostly by inward active Na^+ transport across the cornea (Donn et al., 1959). The result was also confirmed by an in vitro experiment. It was also found that when corneas were bathed on both sides with an iso-osmotic solution of KCl (glucose- and bicarbonate-free), the minimal potential difference was observed (0.0 ± 0.5 mV). Thus, in this study, potential differences resulting from ionic concentration and hydrostatic gradients were interpreted wholly as diffusion and streaming potentials respectively. The effect of KCl on tissue

viability and integrity is presented in a subsequent section.

Effect of pH on diffusion potentials

KCl diffusion potentials were measured with the cornea bathed on each surface by KCl solutions with a fixed 10:1 concentration difference. The more dilute solution (solution 1 with $C_1 = 16$ mM) was applied to the endothelial side and the concentrated solution (solution 2 with $C_2 = 160$ mM) was applied to the epithelial side. All solutions were kept isotonic with the tissue, by the addition of sucrose, to prevent convective bulk flow resulting from osmotic gradient. The effect of sucrose on electrode standard potentials and ion activities in this concentration range was negligible (Barry and Diamond, 1970). The pHs of the solutions, being identical on both sides, were varied from 2.5 to 10 with the addition of HCl or KOH. Steady-state potential difference values, normally attained within 15 min following solution changes, were used in the calculations. The effect of pH from 2 to 10 on diffusion potentials is illustrated in Fig. 1. It can be seen that at high pH (~ 3-10) the potential difference is positive with respect to the endothelial side, indicating that the cornea is more permeable to K^+ than to Cl^- ions, while at

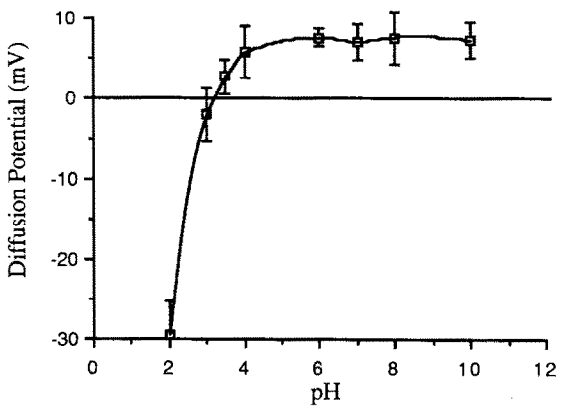

Fig. 1. Effect of pH on KCl diffusion potentials. The diffusion potential resulting from concentration gradient, being 160 mM KCl in the epithelial side and 16 mM KCl in the endothelial side, was measured as a function of pH (abscissa). The ordinate is the diffusion potential at a given pH. The reversal of sign of the potential occurs at the isoelectric pH of 3.2. Bars indicate 1 S.E.M., $n = 6$.

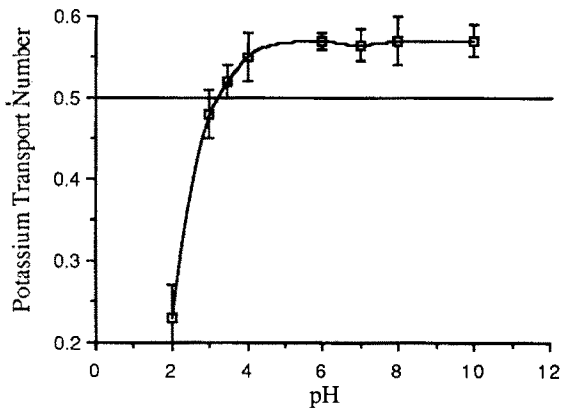

Fig. 2. Effect of membrane charge on transport number of potassium (t^K). The t^K (abscissa) was calculated from KCl diffusion potential according to Eqn. 9. The diffusion potentials, measured for a 10:1 KCl concentration gradient, were taken from those presented in Fig. 1. The abscissa indicates pHs of the bathing solutions. Note that the cornea behaves as if it is negatively charged at high pH and reverses its sign at pH below the isoelectric point. Bars indicate 1 S.E.M. $n = 6$.

low pH (~ 2-3) there was a reversal in polarity. The cornea showed no ionic discrimination against K^+ or Cl^- at pH 3.2 ± 0.2 (n = 6), where $\Delta E_m = 0$. At this pH the cornea is electrically neutral and the pH is called the isoelectric pH.

Unlike streaming potentials, diffusion potentials offer an advantage in that they permit calculation of apparent relative permeability coefficients or transport numbers in the case of conducting ions such as K^+ and Cl^-. Thus, a better demonstration for membrane permselectivity to these ions can be obtained by plotting transport number as a function of pH. This presentation is shown in Fig. 2. As with membrane potential difference, the effect of pH on transport number of K^+ (t^K, calculated from Eqn. 9) is quite similar, but the opposite holds for Cl^-. At any degree of selectivity, the values of t^K can range anywhere from 0 (no permeation for K^+) to 1 (only selective to K^+). As can be seen from Fig. 2, the t^K was virtually independent of pH between pH 4 and 10. These values, close to 0.6, indicate an estimated permeability ratio of 6:4 for K^+ and Cl^-. Below pH 4 the t^K decreased with decreasing pH and below pH 3.2 t^K was actually less than t^{Cl}. This result suggests that the cornea is negatively charged and selectively permeable to cation at high pH but

became positively charged and selectively permeable to anion at pH below the isoelectric point. The result also suggests that the negatively charged groups that govern the permselectivity of the cornea appear to have a pK_a of approximately 3.7.

In a number of epithelia, similar membrane selective properties were observed. With regard to the isoelectric point which was determined mainly by streaming potential measurement, a value of 3.1 was obtained for gall bladder (Moreno and Diamond, 1974), 3.0 for gastric mucosa (Bajaj et al., 1977), 2.7 for small intestine (Smyth and Wright, 1966), 3.5 for urinary bladder (Lipman et al., 1966), and 5.1 for skin (Amberson and Klein, 1928). Although the chemical identity of the membrane charges cannot yet be stated unequivocally, it is suggested that (Shutten, 1981) at physiological pH this charge results from a greater number of ionizable protein amino acid residues (i.e. carboxylic acid groups) as compared to the protonated amine groups, which line the transport pathway. In the case of the cornea, additional charges may result from acidic and basic groups of stromal protein collagen and sulfate groups of the proteoglycans (Davson, 1980), the two major constituents of all the solid matter of the cornea.

Effect of ionic strength on diffusion potentials

To study the effect of ionic strength on membrane permselectivity, ionic concentrations of the bathing solutions were varied, but in all experiments, a constant 10:1 concentration gradient was maintained. All test solutions were made isoosmotic with the equivalent replacement of sucrose. The KCl concentrations (mM) of the test solutions in each experiment were as follows (epithelial/endothelial): (a) 160/16, (b) 64/6.4, (c) 25.6/2.56, and (d) 10.24/1.024, respectively. No buffer electrolyte was added and the final pHs of all tested solutions were ~ 6.0. The result, shown in Fig. 3, indicates that as the ionic strength of the bathing solution decreases (from 160/16 to 10.24/1.024 mM) membrane selectivity to cation increases, approximately from 55 to 65% as compared to the anion. As mentioned earlier, the cornea would give no discrimination to either ions and absolute discrimination to an anion if t^K values of 0.5 and 1 are obtained. The basis for this

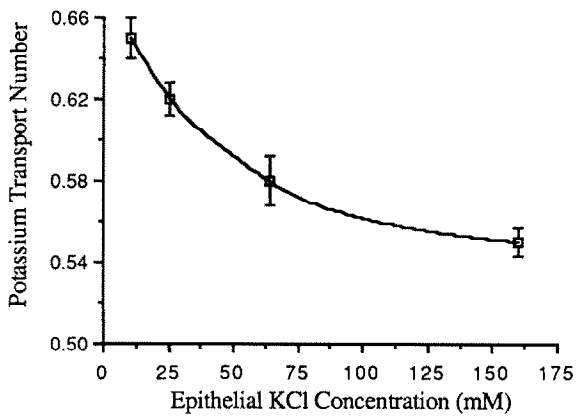

Fig. 3. Effect of ionic strength on transport number of potassium (t^K). The t^K was calculated from KCl diffusion potential according to eqn. 9. A 10:1 KCl concentration gradient was maintained throughout the experiment. All test solutions were adjusted isosmotically at 290–295 mOsm with the addition of glucose. The KCl ionic strength was varied by adjusting KCl concentrations (mM) in the epithelial and endothelial sides as follow: (a) 160/16, (b) 64/6.4, (c) 25.6/2.56 and (d) 10.24/1.024, respectively. Bars indicate 1 S.E.M., $n = 6$.

permselectivity phenomenon has been described as a result of electrostatic potential barrier generated by fixed charge groups within the membrane, the so-called Donnan exclusion effect (Lakshminarayanaiah, 1969; Helfferich, 1962). The lowering in ionic strength of the bathing solution causes a lesser degree of electrostatic shielding of these charged groups and this leads to an increase in effective charge density which demonstrated itself in a greater membrane permselectivity.

Effect of ionic strength on electrical resistance

Although the diffusion potential experiments show an increase in the relative permeability of cation to anion, they do not provide information on the absolute permeability of each ion. Thus, the increase in cation selectivity of the membrane can be as a result of the increase in absolute permeability of the cation, a decrease in absolute permeability of anion, or a combination. The experiment based on resistance measurement provides information about absolute ionic permeabilities since resistance is the sum of the partial ionic resistances and since the partial resistance of an ion is inversely proportional to its absolute permeability.

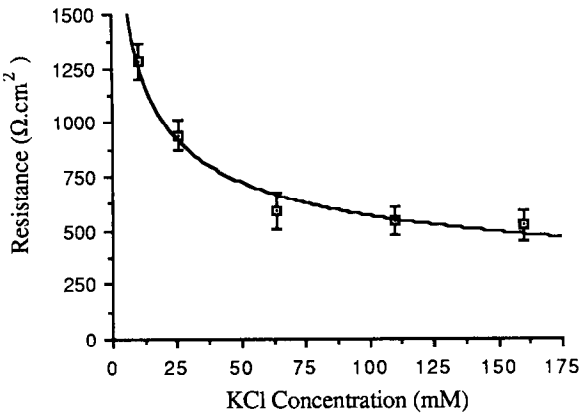

Fig. 4. Effect of ionic strength on membrane resistance. Electrical resistance of the cornea was measured in isotonic KCl solutions having different KCl ionic strengths (160, 110, 64, 25.6 and 10.24 mM). Identical bathing solutions were used for each determination and the pHs of all solutions were at ≈ 6.0 In determining the resistance, a two-paired electrode system (Ag/AgCl) was used. One pair was used to inject current pulses and the other used to detect the potential drops. Bars indicate 1 S.E.M., $n = 6$.

In this study, the resistances of the cornea were measured in different KCl solutions varying in ionic strength (160, 110, 64, 25.6 and 10.24 mM, respectively). The osmolarities of the solutions were adjusted as described previously. All measurements were completed within 5 min after mounting of the cornea. The effect of ionic stength on corneal resistance is shown in Fig. 4. Similar to previous experiments, lowering in ionic strength of the bathing solutions causes a significant increase in membrane resistance. Considered from the magnitude of the change of these resistances (522 $\Omega \cdot cm^2$ at 160 mM to 1,284 $\Omega \cdot cm^2$ at 10.24 mM), it is clear that not only the total permeabilities of the ions decrease but also the absolute permeability of each ion. The effect of increasing membrane resistance can be attributed to greater electrostatic potential barrier to permeation of ions as a result of a lesser degree of electrostatic shielding when ion concentrations in the membrane are reduced.

Effect of pH on streaming potentials

To confirm the value of the corneal isoelectric point obtained earlier, an independent study based on streaming potential measurement was carried out. In contrast to diffusion potentials which are caused by the presence of a concentration gradient and the subsequent flow of the solutes, streaming potential is caused by flow of the solvent under a hydrostatic or osmotic pressure gradient. The potential is developed as a result of induced-convective flow of solutes through a permselective membrane. Preliminary work conducted in this laboratory demonstrated that streaming potential measurements based on an osmotic gradient is rather difficult due to instability and a relatively small magnitude of the potentials.

In the present paper, all measurements were based on a hydrostatic pressure gradient and the solutions bathing the cornea were 160 mM KCl, identical on both sides. First, the effect of pressure gradient on the magnitude of streaming potential was studied. This study was performed in solutions that contained no additional buffer electrolytes. The pressure gradient, generated by a regulated pump with a pressure gauge, was externally applied to both sides of the membrane. The pressure head was always higher on the endothelial side to maintain natural curvature of the cornea. The result of this study, shown in Fig. 5, demonstrates an apparent linear relationship between streaming potential, e.g., being positive on the epithelial side, and pressure gradient with an average proportionality constant of 3.5 mV/atm. This

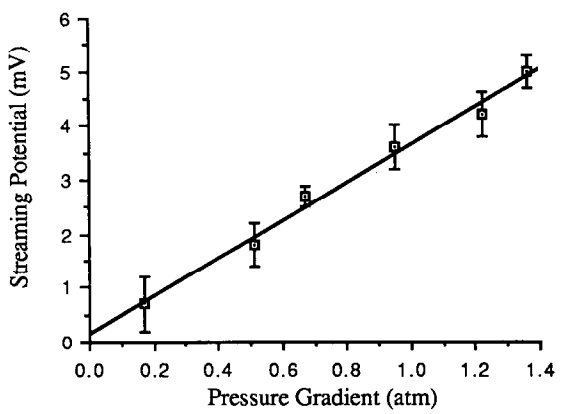

Fig. 5. Effect of hydrostatic pressure gradient on streaming potentials. Bathing solutions on the epithelial and endothelial sides were identical (160 mM KCl). The streaming potentials resulting from pressure gadients, being higher on the endothelial sides, were measured. A positive potential means epithelial side positive. Bars indicate 1 S.E.M., $n = 4$.

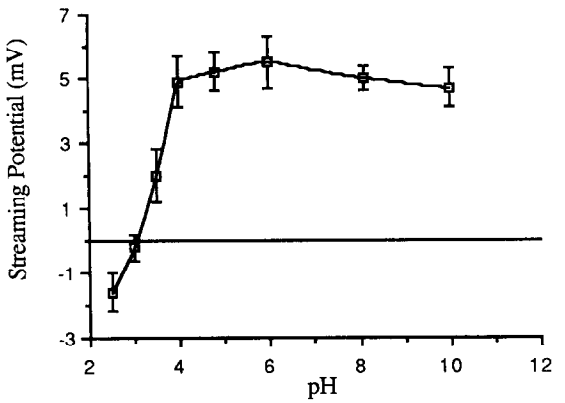

Fig. 6. Effect of pH on KCl streaming potentials. Bathing solutions on the epithelial and endothelial sides were identical (160 mM KCl). The pressure gradient was kept constant at 1.3 atm throughout. A positive potential means epithelial side positive. Bars indicate 1 S.E.M., $n = 6$.

result is in good agreement with theoretical prediction (Katchalsky and Curran, 1967) which states a direct relationship between the hydrostatic pressure gradient (ΔP) and the rate of water flow across the membrane (J_v), according to the equation; $J_v = L_p \cdot \Delta P$, where L_p is the hydraulic filtration coefficient.

For the effect of pH on streaming potentials, a constant pressure gradient of 1.3 atm was used throughout. This value was selected since it was high enough to give good reliability in potential measurement, but at the same time not too high to cause potential damage of the tissue. The pHs of the solutions were adjusted similarly as in diffusion potential experiments. The result, illustrated in Fig. 6, shows striking similarity between the titration curves obtained from the two independent experiments. From Fig. 6, an isoelectric point of 3.1 ± 0.2 ($n = 6$) was obtained for the membrane charge responsible for streaming potentials in the cornea. Again, the evidence suggests that a change in bathing solution pH altered the ratio of negatively charged to positively charged groups in the membrane, resulting in a change in fixed charge density, which manifested itself as a change in permselectivity. This result clearly substantiates the existence of fixed charged groups and their important role in regulating membrane permselectivity.

Tissue viability and integrity test

Tissue viability and integrity were checked due to their importance in determining the overall transport characteristics of the membrane. At the end of each experiment, the bathing solutions were replaced with freshly prepared GBR solutions, and the transmembrane potential differences and resistance were determined at 37 °C. Potential differences, developed when two identical solutions were placed on both sides of the membrane, indicate active ion transports and thus the viable state of the tissue. Membrane resistance, on the other hand, indicates membrane permeability and thus it was used as an indication of tissue integrity or damage. The result of the potential difference measurement is shown in Fig. 7. It can be seen that minimal potential difference was developed over the initial period when the cornea was placed in an isotonic KCl solution. However, as soon as the solution was replaced with the GBR solution, potential differences in the range of 15–20 mV, being negative on the epithelial side, were observed. The potential was maintained and gradually fell to the baseline level as soon as the KCl solution was resubstituted. In

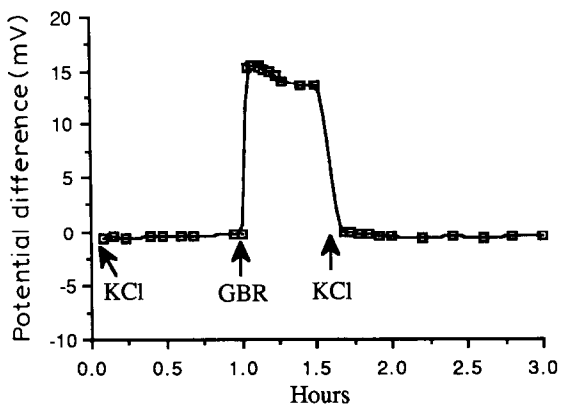

Fig. 7. Transmembrane potential difference in isotonic KCl and GBR solutions. The corneas obtained after diffusion and streaming potential experiments were placed alternately in isotonic KCl and GBR solutions and the potential differences were measured as a function of time. Potential difference developed in an identical bathing solution indicates active ion transport, and thus the viable state of the tissue. The result demonstrates the maintainance of tissue viability and the absence of active transport in the two electrokinetic potential experiments.

an independent study (result not shown), when only GBR solution was used, the potential difference in the range 20–25 mV was developed and maintained for as long as 6 h. Further investigations in this laboratory indicated that this potential was accounted for almost entirely by Na^+ active transport. These studies thus clearly demonstrate maintenance of tissue viability and the absence of active transport in diffusion and streaming potential experiments.

In the tissue integrity test, membrane resistances in GBR solutions were measured for the corneas after diffusion and streaming potential experiments as well as corneas that had been freshly excised. In freshly excised corneas, the average resistance for 6 determinations was $891 \pm 57\ \Omega \cdot cm^2$ while those in diffusion and streaming potential experiments were 807 ± 44 and $753 \pm 61\ \Omega \cdot cm^2$, respectively. The slight decrease in membrane resistances in these two experiments may be attributed to some change in tissue integrity during the experiments or a time-dependent nature of the corneal resistance. It was also found that corneal resistance determined in vitro, and in GBR solution, decreased slightly with time and the magnitude of this change was comparable to the change observed in the previous two experiments.

Conclusions

A permselectivity study based on two simple electrical measurements, namely diffusion and streaming potential measurements, was conducted in the cornea. The study, based on the effect of pH on these potentials, showed the similarity between the pH–potential profiles and the titration curve of an amphoteric substance, suggesting that the cornea contains both acidic and basic charge groups which are probably responsible for the electrostatic potential barrier and the observed differences in permeabilities between cation and anion. At physiological pH and above the isoelectric point, the cornea carries a net negative charge, is relatively less permeable to anions, and functions as an ion-exchange membrane for cations. At lower pH, both charge groups become protonated so that the cornea carries a net positive charge and has the reverse effect on permeation of the ions. Lowering the ionic strength of the bathing solution caused an increase in membrane-effective charge density due to a lesser degree of electrostatic shielding. This results in a significant increase in both membrane resistance and selectivity. Tissue viability and integrity were evaluated based on transmembrane potential difference and resistance. The result indicated the preservation of these two membrane properties during permselectivity studies.

This study has demonstrated the significance of membrane permselectivity to transport of charged solutes in biological system. This aspect of membrane transport has not been fully recognized in the general approach to transport problems. As understanding of membrane phenomena progresses, it becomes necessary that this traditional approach is reexamined and a greater awareness to complexity of the problem is given. In addition, this study has also shown that modification of membrane permselectivity can be made, to some degree, by controlling the degree of protonation and electrostatic shielding of the charge groups of the membrane. The knowledge from this study may provide strategies to transepithelial delivery of drugs especially those with charges, i.e. peptides and proteins. In the case of peptides and proteins, the problem of barrier permselectivity may be much more complex due to higher and varying degrees of charge densities of these compounds. Furthermore, due to limited dimensions of membrane transport pathways, the problem of molecular size may additionally become a dominant factor in determining the final transport process.

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