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Topical Review

Ion Selectivity of Epithelial Na Channels

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Introduction

Koefoed-Johnsen and Ussing (1958) first pointed out that the outer (apical) membrane of the frog skin is selectively permeable to Na ions, while the inner (basal-lateral) is selective for K ions. They deduced that this asymmetry underlies the ability of the epithelium to sustain net transfer of Na from a dilute solution (the pond) to a concentrated solution (the blood). Na enters the epithelial cells across the apical membrane passively and is then actively transported across the basal-lateral membrane by the Na-K pump. Since the basal-lateral membrane is much more permeable than the apical membrane to K, most of the K pumped into the cell recirculates across this membrane.

The selectivity of the apical membrane for Na is conferred by the presence of ion channels that conduct Na much more readily than K. These epithelial Na channels seem to be unique to epithelia and have been observed in the amphibian skin and urinary bladder, the mammalian collecting tubule, colon and urinary bladder, and other tight, Na-reabsorbing epithelia. The number of these channels conducting Na across the apical membrane determines to a large extent the transepithelial transport rate. The number of channels is in turn under the control of salt-retaining hormones, including aldosterone and anti-diuretic hormone. More general reviews of these channels and their hormonal control can be found elsewhere (Lindemann, 1984; Palmer, 1986*a*; Saiban-Sohraby & Benos, 1986; Eaton & Hamilton, 1987). Here I will first discuss the basic transport and selectivity properties of the epithelial Na channels. Then I will speculate on the molecular basis for this selectivity and present a simple working model to account for the available information.

Key Words Na transport · apical membrane · ion channels

Selectivity Properties of the Na Channels

Lindley and Hoshiko (1964) made quantitative estimates of membrane selectivity in the frog skin by measuring changes in transepithelial voltage with ion substitutions. They reported a permeability ratio for Na/K of about 20 : 1. It subsequently became apparent that the apical surface contains Na-selective ion channels (Lindemann & Van Driessche, 1977) arranged in parallel with paracellular pathways (Ussing & Windhager, 1964; DiBona & Civan, 1973) and other apical membrane ion conductances (Hviid-Larsen and Kristensen, 1978; Zeiske & Van Driessche, 1979; Palmer, 1986*b*). Thus the selectivity of the Na channels themselves might have been underestimated by Lindley and Hoshiko.

Other laboratories confirmed in the toad urinary bladder that the apical membrane was highly selective for Na over K. Gatzy and Clarkson (1965) found that, although the apical membrane of this tissue did not behave as an ideal Na electrode, the change in transepithelial voltage with changes in mucosal Na concentration were similar when Na was replaced by K or by a presumably impermeant cation such as tetraethylammonium. They concluded that K, as well as TEA, did not permeate the apical membrane to a significant extent. This was confirmed by measurements of K fluxes across this membrane. Finn and Nellans (1972) reported that when cells were loaded with ^{42}K, most of the isotope washed out across the basal-lateral rather than the apical membrane. Robinson and Macknight (1976) found that when the K isotope was present in the mucosal solution that a negligible amount of the cell K was labeled even after a 1-hr incubation.

More quantitative estimates of the selectivity of the channels *per se*, rather than of the membrane or epithelium as a whole, have been facilitated by the use of amiloride, a guanidinium-based, K-sparing diuretic that reversibly blocks epithelial Na chan-

nels with apparent inhibition constants of 1 μM or less (Benos, 1982).

Benos, Mandel and Simon (1980*b*) measured amiloride-blockable short-circuit current across the frog skin in the presence of either K or Na in the mucosal solution and found no detectable channel-mediated K current. They concluded that the selectivity of the channels for Na over K was at lest 100 : 1. A similar result was obtained by Palmer (1982) from measurements of amiloride-sensitive currents under conditions of an inwardly directed Na gradient and an outwardly directed K gradient across the apical membrane of the K-depolarized toad urinary bladder. In that study, apparent amiloride-sensitive K currents across the apical membrane were detected, although these currents were quite small. When converted to permeabilities, these measurements implied a Na/K selectivity of 500 to 1000.

A high selectivity for Na over K was also confirmed in recent measurements of currents through individual Na channels in the apical membrane of the rat cortical collecting tubule (Palmer & Frindt, 1986*a*). The permeability ratio estimated from the reversal potential of single-channel currents in excised patches was at least 10 : 1. More precise estimates were difficult due to the small size of the single-channel current of the 5 pS channels.

On the other hand, there have been a number of reports of amiloride-sensitive channels in epithelia which, at least under certain circumstances, have a relatively poor selectivity for Na over K. These include the toad skin just after moulting, the tadpole skin, the urinary bladder of rabbits on a high salt diet, and toad kidney cells in culture.

Katz (1978) studied the amiloride sensitivity of the conductance and short-circuit current of the toad skin throughout the moulting cycle. He found that when the skin was mounted just after the stratum corneum was shed, transepithelial conductance could be reduced by amiloride even when the mucosal surface was bathed in a Na-free, K-containing solution. This effect of amiloride was apparently not due to contamination with Na or to block of the paracellular pathway.

Hillyard, Zeiske and Van Driessche (1982), studying the fluctuations in short-circuit current across the skin of the larval bullfrog, found that amiloride enhanced the spontaneous Lorentzian noise seen with K in the mucosal solution and induced Lorentzian noise in the presence of Na. The authors interpreted these findings as indicating the existence of channels that were sensitive to amiloride but which did not discriminate strictly between Na and K. They suggested that these channels may be precursors of the mature channel type which shows Lorentzian noise only in the presence of both Na and amiloride in the mucosal bath.

Lower selectivity ratios were also obtained by Lewis and Wills (1983) in urinary bladders from rabbits on a standard (high Na) diet. They measured the emf of the amiloride-sensitive pathway and found values considerably smaller than the Na equilibrium potential, which was estimated using conventional and ion-selective microelectrodes. The reversal potential was not measured directly but was extrapolated from measurements of membrane current and slope conductance. Thus if the current-voltage relationship of the amiloride-sensitive pathway shows Goldman-type rectification (Fuchs, Hviid-Larsen & Lindemann, 1977), the reversal potential, and hence the selectivity, could be underestimated. However, the difference between the estimated reversal potential (23–26 mV) and the Na equilibrium potential (about 70 mV) is too large to be accounted for by this discrepancy. Calculated permeability ratios of Na/K were 2.6 for animals on a normal diet and 9 for animals on a low Na diet. The authors attributed the difference to two populations of channels, those with the higher selectivity being activated by aldosterone, which is elevated in animals on a low Na diet.

Sariban-Sohraby et al. (1984) provided the first measurements of single-channel currents through amiloride-sensitive Na channels that were reconstituted from cultured toad kidney cells (A6 cell line) into planar lipid bilayers. The selectivity of these channels was also low. The permeability ratio from reversal potentials of current-voltage plots was 2 : 1, while conductance ratios for Na/K were 2.8 : 1. Unfortunately, since no direct measurements of selectivity of the intact membrane in these cells was available, it is not clear whether the low selectivity results from changes occurring during the continuous culture of the cells or if the channels might have been modified during the process of homogenization and reconstitution.

Some additional insight on this point was provided by recent experiments with the A6 cell line using the patch clamp technique (Hamilton & Eaton, 1985, 1986). Two types of Na channels were observed, both of which were sensitive to amiloride. The first type had a relatively high conductance (7 to 10 pS) and a relatively low selectivity for Na/K (3 or 4 : 1). It was seen predominantly in cells grown on plastic dishes, where the channel density is low. The second type had a lower conductance, slower spontaneous kinetics and was apparently much more selective for Na, as no reversal currents could be detected under biionic conditions in which K would carry the reversed currents. These channels were seen predominantly in cells grown on per-

Table 1. Selectivity for Na over K

Tissue	Na/K	Method	Reference
Frog skin	20 : 1	Change in transepithelial voltage	Lindley & Hoshiko (1964)
Frog skin	>100 : 1	Amiloride-sensitive current	Benos et al. (1980*b*)
Toad bladder	500 : 1	Amiloride-sensitive currents	Palmer (1982)
Rabbit bladder			
Normal diet	2.6 : 1	Apical membrane emf	Lewis & Wills (1983)
Low Na diet	9 : 1	Apical membrane emf	Lewis & Wills (1983)
A6 cells	2–3 : 1	Single-channel reversal potential (bilayers)	Sariban-Sohraby et al. (1984)
A6 cells			
Impermeable support	3–4 : 1	Single-channel reversal	Hamilton & Eaton
Permeable support	>20 : 1	potential (patch clamp)	(1985, 1986)
Rat cortical collecting tubule	>10 : 1	Single-channel reversal potential	Palmer & Frindt (1986*a*)

meable supports in which the channel densities are high. The relationship between these two channel types and those seen by Sariban-Sohraby et al. (1984) is still somewhat unclear, as the reconstituted channels were obtained from cells grown on permeable supports and yet had low selectivity. In addition, the reconstituted channels exhibited a surprisingly large range of single-channel conductance, from 4 to 80 pS in 200 mM NaCl.

The molecular processes underlying the transitions from low to high selectivity are not understood. It is possible that two or more gene products are involved, with the gene coding for low selectivity channels being the predominate one transcribed in early development, moulting, low aldosterone and in cell culture under certain conditions. It is also possible that one channel type might be a product of the other, for example as a covalent modification or a proteolytic cleavage.

In summary, epithelial Na channels exhibit, under most circumstances, a remarkable ability to distinguish between Na and K ions, whether the permeabilities to the two ions are measured from tracer fluxes, macroscopic ionic currents, or single-channel current reversal potentials (*see* Table 1). Under certain conditions this selectivity may be impaired. The rest of the review will focus on the high selectivity Na channels as seen in toad urinary bladder, adult frog skin and the mammalian cortical collecting tubule.

The selectivity of the channel for ions other than Na and K can be summarized by the rule that ions smaller than Na permeate the channel, while ions larger than K (or Na) do not. Permeant ions are Li and H. Impermeant ions, or ions that have immeasurably small permeabilities, include Rb, Cs and all the small "organic" cations tested, such as derivatives of ammonium or guanidinium.

Several investigators have shown that Li can substitute for Na in carrying current across the apical membrane of a number of tight epithelia (Herrera, Egea & Herrera, 1971; Sarracino & Dawson 1979; Benos et al., 1980*b*; Macknight & Hughes, 1981; Palmer, 1982). Sarracino and Dawson (1979) found that Na and Li carried comparable currents across the turtle bladder. Similar results were obtained in frog skin (Benos et al., 1980*b*). These results are complicated by at least two factors. First, ions have to cross both the apical and basal-lateral membranes to contribute to the steady-state current. Second, intracellular Li apparently has some toxic effects (Sarracino & Dawson, 1979). To get around these difficulties, Palmer (1982), using the K-depolarized toadbladder, made rapid substitutions of mucosal Li for Na under conditions where the cell and mucosal Na concentrations were approximately equal. It was found that the condition for zero current flow across the apical membrane was achieved with Na concentrations 1.3-fold higher than the Li concentration, implying a selectivity for Li over Na of 1.3 : 1. Finally, in patch clamp studies of Na channels in rat CCT the single-channel conductance for isotonic Li was 7.4 pS, 50% higher than that for isotonic Na (4.9 pS) (*see* Table 2). Thus, the channel appears to have a small selectivity for Li over Na whether the selectivity is measured as a conductance ratio or a permeability ratio.

Protons also appear to go through the Na channel. Palmer (1982) measured an amiloride-sensitive current that was apparently carried by protons when the mucosal bath of the toad bladder was acidified to pH 5 or below. Subsequently this current was found to be a saturable function of mucosal concentration with half-maximal transport rates at about 0.1 mM (Palmer, 1984). Thus protons appear

Table 2. Selectivity for Li over Na

Tissue	Li/Na	Method	Reference
Turtle colon	~1 : 1	Steady-state short-circuit current	Sarracino & Dawson (1979)
Frog skin	1.1 : 1	Steady-state short-circuit current	Benos et al. (1980*b*)
Toad bladder	1.3 : 1	Current reversal under short-circuit conditions	Palmer (1982)
Rat cortical collecting tubule	1.5 : 1	Single-channel conductance ratio (patch clamp)	L.G. Palmer & G. Frindt (*unpublished*)

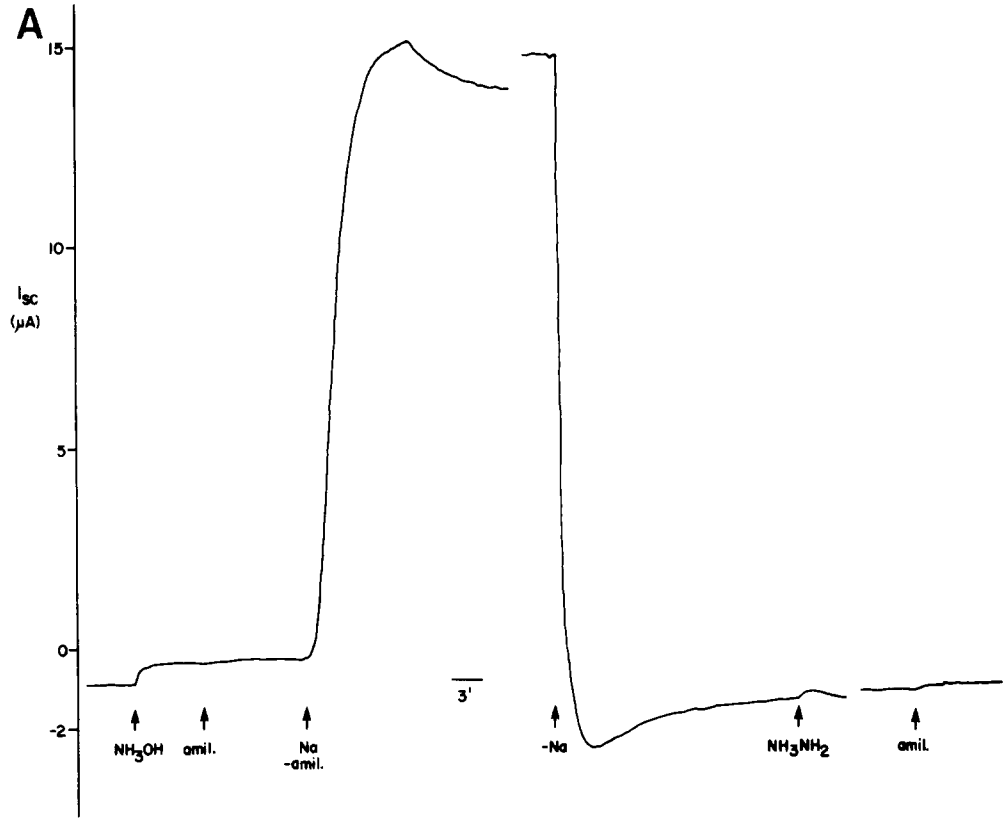
to bind to a site within the lumen of the channel on their way through. Saturation of this binding site limits the amount of current that can be carried by H and may also account for the block of Na conduction through the channels observed at acid pH (Palmer, 1984). Na probably associates with the same site as it passes through the channel, contributing to saturation of the Na current with increasing Na concentrations as observed first by Frazier, Dempsey and Leaf (1962) in the toad bladder and subsequently by many other investigators. Although half-maximal Na currents were obtained at 20 to 30 mM mucosal Na, part of this effect may be due to downregulation of channel number by mucosal Na (Van Driessche & Lindemann, 1979), and the concentration required for half-maximal conductance through individual Na channels may be higher. Van Driessche and Lindemann (1979), using noise analysis, found no detectable saturation of single channel currents in the frog skin at Na activities up to 60 mM. Palmer and Frindt (1986*a*) found half-maximal conductance at about 75 mM Na in the rat CCT using the patch clamp technique. Olans, Sariban-Sohraby and Benos (1984), on the other hand, found half-maximal conductance at 18 mM Na in Na channels reconstituted from A6 cells. This value is similar to that needed to half-saturate macroscopic Na currents in that tissue. In any case, Na appears to bind to a cationic binding site within the channel much more loosely than H. Thus, although protons have a calculated permeability through the channel that is higher than that to Na (Palmer, 1984; 1985), the maximal conductance to Na is much larger than that to H.

Ions which do not penetrate the channel to any appreciable extent include hydroxyammonium (NH_3OH) and hydrazinium (NH_3NH_2) (Palmer, 1982). This property of the channels has also been confirmed in patch-clamp studies of rat CCT (Fig. 1). This finding is of interest in that it clearly distinguishes the conduction path in the epithelial Na channel from that of the excitable Na channel, at least in the node of Ranvier of the frog, where both NH_3OH and NH_3NH_2 are conducted almost as well as Na (Hille, 1975). The lack of permeability of these ions also has implications for estimating the size of the pore, as discussed below.

It should be pointed out that the term "selectivity" is somewhat ambiguous. There are at least two different ways to determine a selectivity of an ion channel. One is to compare the conductance of the channel in the presence of different ions. Another is to measure the reversal potential of the channel under biionic conditions with the two ions present on opposite sides of the membrane. The selectivities measured by these two methods are not necessarily equivalent, as has been discussed in detail elsewhere (*see* Läuger, 1973). In the case of the epithelial Na channel, the selectivity, at least among the ions discussed above (with the exception of H), is at least qualitatively the same whether conductances or permeabilities are compared. The basic conclusions that the channel mildly prefers Li to Na but strongly prefers Li or Na over K and NH_3OH is independent of which measurement is used.

One question that has received little attention is whether water can go through the channel. This cannot be resolved in a straightforward way by looking at the effect of blocking the channels on transepithelial water flow, since water flow across the rest of the epithelium is too large. An indirect way of looking at water flow through ion channels is to impose osmotic gradients across the membrane and measure their effects on ion flow via streaming potentials. In this way it has been possible to estimate the ratio of water molecules/ions transported by gramicidin channels and reconstituted K channels from sarcoplasmic reticulum (Levitt, Elias & Hautman, 1978; Rosenberg & Finkelstein, 1978; Miller, 1982).

To apply this technique to epithelial Na channels, K-depolarized toad bladders were mounted



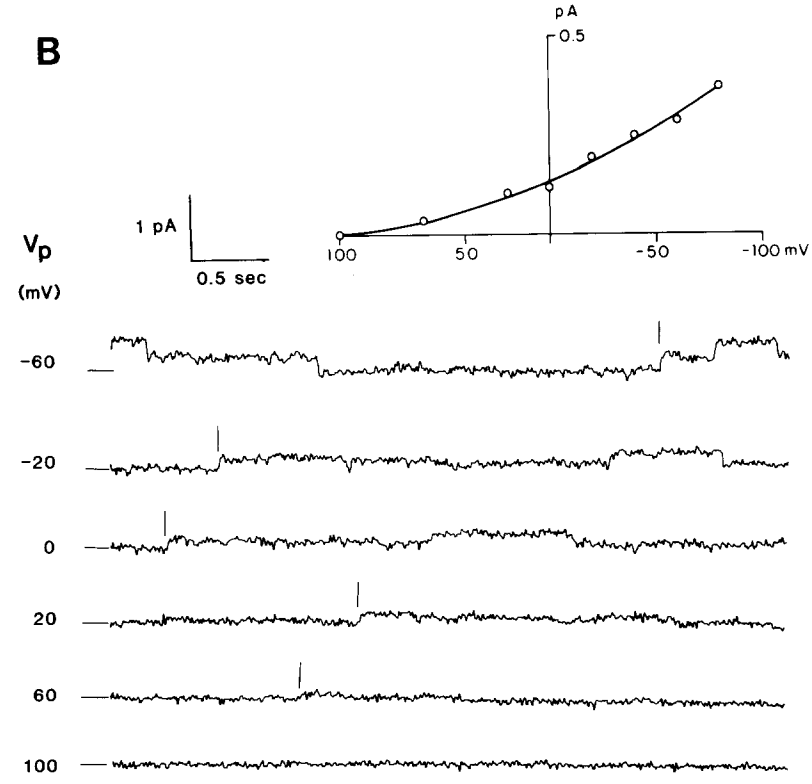


Fig. 1. Impermeability of the epithelial Na channels to small organic cations. (*A*) Toad urinary bladder. Short-circuit currents were measured across the K-depolarized toad bladder with various cations in the mucosal solution, with and without amiloride. While there is a large current carried by Na, only small currents are produced by addition of NH_3OH or NH_3NH_2 to the medium, and these currents are not abolished by amiloride. (Reproduced with permission of the publisher from Palmer, 1982) (*B*) Rat cortical collecting tubule. Single channel recordings of Na channel activity were made from an inside-out patch as described by Palmer and Frindt (1986). The pipette contained NH_3OH Cl, and the bath contained NaCl. Upward current transitions, as marked by the vertical lines, correspond to channel openings. Single-channel currents are observed with positive voltages in the pipette relative to the bath, driving Na through the channel. No reversal of the currents was seen even with pipette voltages of 100 mV, indicating that the conductance of the channel to NH_3OH was immeasurably small

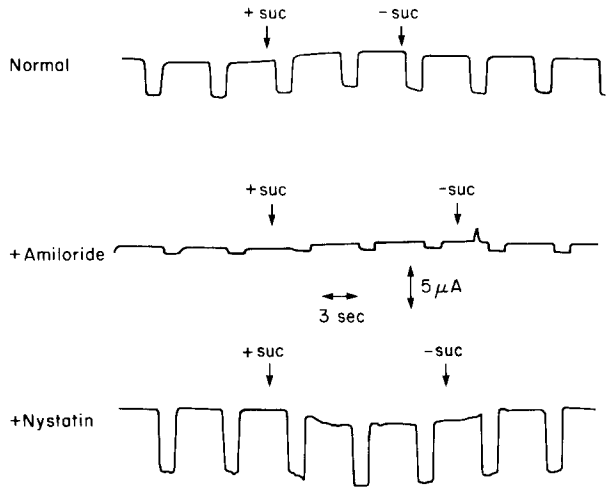


Fig. 2. Insensitivity of Na movement through the channels to an osmotic gradient. The short-circuit current across the K-depolarized toad bladder was measured. Brief changes of 10 mV in the clamping voltage were used to compute the transepithelial conductance. Ouabain (5 mM) was added to the serosal side to increase the Na activity of the cytoplasm, and a sudden decrease in the mucosal Na concentration to 25 mM was made to achieve condition of nearly zero short-circuit current. Under these conditions, the mucosal solution was rapidly exchanged for one containing 1 molal sucrose. The control solution was returned after about 1 min. There was a small but consistent increase in the short-circuit current in response to the sucrose. In the presence of amiloride (10 μM) the current remained near zero, but the conductance decreased. Application of sucrose resulted in a similar small increase in short-circuit current, which was presumably carried across the paracellular pathway. Addition of nystatin (5 μg/ml) increased the tissue conductance, presumably by forming cation-selective pores in the apical membrane. Application of sucrose under these conditions resulted in a negative short-circuit current which could have resulted from the interaction of permeant ions with water flowing out of the cells through the nystatin pores

and their cells loaded with Na by application of serosal ouabain (Palmer, 1982). When the net Na current had dropped to very low levels, 1 molal sucrose was added to the mucosal solution. The reduction in water activity in this compartment should pull water out of the epithelial cells. If water is passing through the apical Na channels, it should pull Na with it, creating, at least transiently, a current opposite in direction to that seen in a normally transporting tissue. As seen in Fig. 2, mucosal sucrose addition produced a change in current that was small in magnitude and opposite in direction to that predicted from this line of reasoning. Furthermore, blockade of Na channels with amiloride decreased the total tissue conductance but did not affect the small change induced by hyperosmotic sucrose. For a positive control, the apical membrane was doped with nystatin, a polyene antibiotic which forms rather large pores in cholesterol-containing lipid bilayers. These pores are cation selective when the molecule is added to one side only in planar lipid bilayers (Cass, Finkelstein & Krespi, 1970) as well as epithelia (Lewis et al., 1977). Here mucosal sucrose gave rise to a current in the direction expected of a streaming potential developing across the pore.

These findings must be interpreted with caution, as the imposition of an osmotic gradient across this biological membrane may have multiple effects which could mask a small amiloride-sensitive streaming potential. Nevertheless, the toad bladder is fairly well suited to this type of experiment since there are no other major ion conductance pathways present under these conditions, and the permeability to water is, in the absence of antidiuretic hormone, quite low, minimizing the dissipation of the osmotic gradient.

With this caveat in mind, there are at least two possible interpretations. First, water and Na could move through the Na channel without interacting appreciably. This would be the case, for example, in a wide pore in which the water molecules could easily pass around the Na ions on their way through. A second interpretation, which seems to be much more likely in view of the high selectivity of the channel, is that the pore is too narrow to let the water molecules through at all.

Molecular Basis for Selectivity

Outer Binding Site

Many cations which do not permeate the channel can block the permeation of Na in a voltage-dependent fashion (Palmer, 1984, 1985). This implies the existence of a binding site or sites within the electric field of the pore which can be occupied either by Na or by other ions which act as competitive inhibitors of transport. These ions include alkali metal ions such as K, Rb and Cs, divalent cations such as Mg, Ca, Sr and Ba and various derivatives of ammonium and guanidinium. Binding of Na to this site could represent the first step in translocation through the channel, while binding of a poorly permeant ion could physically clog the channel and prevent Na from being transported. Interactions between ions and this binding site could contribute to the overall selectivity of the channel. Binding preference of the alkali metal cations is in the order H > Na > K > Rb > Cs. This order is consistent with binding to a high field-strength site according to the theory of Eisenman (*see below*). The apparent acid dissociation constant (pK_a) of this site implies the possibil-

ity that the site contains one or more carboxyl residues that are ionized at physiological pH. Access to this site appears to be restricted to cations with an ionic diameter of less than 5 Å (Palmer, 1985). The site might also be the locus of interaction of amiloride with the channel.

Main Selectivity Filter

The outer binding site, however, clearly does not account for the main selectivity characteristics of the channel, namely the high selectivity for Na over K and the impermeability to small organic cations such as NH_3OH. This discrimination must be accomplished at a site in the channel distal to the outer binding site. Below I will discuss two different, though not necessarily conflicting, hypotheses on how this selectivity might be achieved.

"Eisenman Selectivity"

Eisenman (1962) developed a theory to explain different cation selectivities of glass electrodes. It is based on the idea that selectivity among cations depends on the energy of interaction with negatively charged sites such as a halide or the O^- component of a silicate, carbonate or phosphate. As pointed out by Hille (1975), this energy of interaction can also serve to reduce free energy barriers or maxima as well as to provide free energy wells or minima. The former is most likely to determine selectivity in an ion channel (*see also* Eisenman & Horn, 1983).

Eisenman postulated that the selectivity of a negatively charged site is determined by the electrical field strength of the site, which can be expressed as the reciprocal of the effective radius of the anion. On this basis, he derived 11 possible selectivity sequences for Li, Na, K, Rb and Cs. The selectivity of the epithelial Na channel corresponds to that of sequence XI, which characterizes sites of the highest field strength. This conclusion follows from the finding that Li is the most preferred cation, as sequence XI is the only one that begins with Li. Such a site could be either a relatively weak acid compared to a low field-strength site or could be composed of a cluster of lower field-strength sites spaced closely enough to allow overlap of their electric fields.

The carboxyl group of acetate in solution has a pK_a of about 4.8 and binds cations with sequence XI selectivity (Morf & Simon, 1971). Thus a carboxyl group would be a promising candidate for conferring selectivity at the main filter, as well as at the outer mouth of the channel. The degree of selectivity, as opposed to the sequence, depends in Eisenman's model on the amount of water allowed near the site: The less the hydration, the greater the selectivity. Thus the large Na/K selectivity coefficient might be expected of an environment within the narrow part of the channel in which water is sterically excluded (*see below*). The rather mild preference of the channel for Li over Na in the face of a large preference for N over K can also be accounted for by the Eisenman scheme (*see* Fig. 17 of Eisenman, 1962).

It is less clear whether the very small permeability to organic cations can also be explained on the basis of anion field strength. It is difficult to predict the strength of the coulombic interactions between the site and the asymmetric ammonium derivatives since charge can be distributed between the two parts of the molecule in such a way that the NH_3 group carries a charge that can be either greater than or less than 1 (Hille, 1975). According to Hille (1975) a high field-strength site should bind to small N-based cations with a sequence $NH_3OH > NH_3NH_2 > NH_4 >$ guanidinium, and this is the selectivity sequence found in Na channels from frog node of Ranvier, which also appears to have a high field-strength site. Both NH_3OH and NH_3NH_2 were preferred over K and were conducted nearly as well as Na. Thus additional factors may need to be invoked to explain their lack of conductance through the epithelial Na channel.

Steric Selectivity

Another factor which has been stressed by Hille (1971, 1975) in describing the selectivity of biological channels is the geometry of the pore. Taking the notion of a pore literally, it is clear that such a structure will act as a molecular sieve, so that ions larger than a critical size will not pass through. Whether or not an ion will fit through the pore will depend on the size of its hydration shell and to what extent the shell can be stripped off during passage through the channel. The ability of the epithelial Na channel to discriminate between alkali metal cations (Li, Na) and organic cations (NH_3OH, NH_3NH_2) can be accounted for if ions are completely dehydrated during passage and if the size of the pore at its narrowest point is larger than the crystal radius of Na (1 Å) but too small to accommodate the NH_3OH ion, which is about 3 × 5 Å (Hille, 1975).

The finding that the pore is apparently too small to accommodate water molecules, as discussed above, is consistent with this view of the channel. It is also possible that the size of the pore might be small enough to contribute to the Na/K selectivity. Since the crystal radius of K is about 1.3 Å, the low conductance of this ion could be explained if the

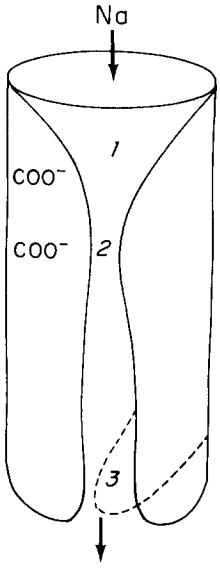


Fig. 3. Working model of the epithelial Na channel. The channel is represented as a pore with a relatively wide (5 Å) mouth (*1*) facing the mucosal solution. The mouth is lined with one or more carboxyl groups which attract small cations to and repel anion from the pore. Deeper into the channel the pore narrows (*2*) such that small organic cations and perhaps also water are excluded. Passage through this region is controlled by another carboxyl group or groups with a high field strength. The channel can also close to all ion traffic, as indicated by the spontaneous current transitions seen in Fig. 1*B*, which correspond to channel openings and closings. This closing is shown as a reversible constriction of the channel near the cytoplasmic end (*3*), although this is completely hypothetical

pore were only slightly larger, or if the pore had to stretch slightly to accommodate the K, but not the Na ion.

Are Carboxyl Groups Involved in Transport through the Channel?

The idea that interaction with high field-strength sites contributes to ion selectivity in the Na channels implies the possibility that one or more carboxyl groups could control passage through the pore. The effects of agents which modify carboxyl groups have been studied by several laboratories with mixed results. Carbodiimides were found to block Na transport by the skin of *Rana temporaria* (Zeiske & Lindemann, 1975) but not by that of other frog species (Benos et al., 1980*a*) or by the toad bladder (Harms & Fanestil, 1977). EEDQ, a more amphipathic carboxyl-modifying reagent, has been shown to inhibit Na transport in toad bladder, and the action of this reagent is protected against by amiloride (Park, Kipnowski & Fanestil, 1983).

The reason for the lack of a consistent result of this kind of experiment may lie in the geometry of the channel. If access to the selectivity filter is through an opening of about 5 Å in diameter, as suggested above on the basis of which ions can block the channel, then the relatively bulky modifying reagents would probably not be able to reach the site of the carboxyl groups either at the outer mouth or at the selectivity filter. The effects of these reagents, when they are observed, may be localized to sites distant from the conduction pathway or, in the case of EEDQ, the site may be reached by a more hydrophobic route.

Summary

Epithelial Na channels are apparently pore-forming membrane proteins which conduct Na much better than any other biologically abundant ion. The conductance to Na can be 100 to 1000 times higher than that to K. The only other ions that can readily get through this channel are protons and Li. Small organic cations cannot pass through the channel, and water may also be impermeant.

The selectivity properties of epithelial Na channels appear to be determined by at least three factors: (1) A high field-strength anionic site, most likely a carboxyl residue of glutamic or aspartic acid residues on the channel protein, probably accounts for the high conductance through these channels of Na and Li and to the low conductance of K, Rb and Cs. (2) A restriction in the size of the pore at its narrowest point probably accounts for the low conductance of organic cations as well as the possible exclusion of water molecules. (3) The outer mouth of the channel appears to be negatively charged and may control access to the region of highest selectivity and may serve as a preliminary selectivity filter, attracting cations over anions.

These conclusions are illustrated by the cartoon of the channel in Fig. 3. This picture is obviously both fanciful and simplified, but its general points will hopefully be testable. It leaves open a number of important questions, including: (a) does amiloride block the channel by binding within the outer mouth? (b) what does the inner mouth of the channel look like, and does this part of the channel contribute to selectivity? and (c) what, if any, are the interactions between the features of the channel that impart selectivity and those that control the regulation of the channel by hormonal and other factors?

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