The Molecular Basis of Movement Through Membrranes

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The Molecular Basis of Movement Through Membranes

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Preface

The last few years have witnessed rapid advances in our knowledge of the way in which a variety of molecules move across cell membranes. This has been made possible partly by the deployment of techniques for sequencing membrane proteins and processing this information to provide space-filling structural co-ordinates, and partly by refinements of conventional biophysical methods. We are grateful to C. F. Phelps, the retiring organizer of the Biochemical Society Symposia, for recognizing the importance of these developments and for joining us in the planning of a Symposium devoted to that topic. On behalf of other members of the Society, we would like further to record our indebtedness to him for his unstinting service over the past 4 years.

This volume, then, is an account of a Symposium organized under the auspices of the Biochemical Society; it was held at St George's Hospital Medical School, London on 19 and 20 December 1984. The Chairmen of the first day were P. D. Mitchell, F.R.S. and P. F. Baker, F.R.S., who presided over a discussion of the passive, and the pumped, movements of H^+, Na^+, K^+ and Ca^{2+}, and of nutrients such as glucose and amino acids. The emphasis was on plasma membranes rather than on mitochondrial or other intracytoplasmic membranes. The sessions on the second day were chaired by P. N. Campbell and C. A. Pasternak and were devoted to a consideration of the movement of peptides and proteins through membranes, of movement through specialized regions such as nuclear 'pores' and communicating junctions, of modulation of ion movement by growth factors and of movement through pores artificially created by toxins, viruses or complement.

We are particularly honoured as Editors of this Symposium to mark a half century of Biochemical Society Symposia. The list of topics covered is extensive and reflects the development of biochemistry as a leading subject in the life sciences. The list of contributing authors is equally impressive and includes scientists of distinction in every branch of the subject.

Finally, in connection with the organization of the meeting at St George's we would especially like to extend our gratitude to the local organisers, M. J. Clemens and J. M. Graham.

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London

Abbreviations

a.h.p.	After-hyperpolarization potential
BCECF	Bis(carboxyethyl)carboxyfluorescein
DG	1,2-Diacylglycerol
DHP	Dihydropyridine
DMSO	Dimethyl sulphoxide
DOC	Sodium deoxycholate
DSS	Disuccinimidyl suberate
EGF	Epidermal growth factor
EGTA	Ethanedioxybis(ethylamine)tetra-acetic acid
EMC	Encephalomyocarditis (virus)
FCCP	Carbonyl cyanide-*p*-trifluoromethoxyphenyl hydrazone
HAU	Haemagglutinating unit
HBS	Hepes-buffered saline
IGF-II	Insulin-like growth factor II
IP3	Inositol triphosphate
LDL	Low-density lipoprotein
MAC	Membrane attack complex
PDGF	Platelet-derived growth factor
PEA	*Pseudomonas* exotoxin A
PIP2	Phosphatidylinositol 4,5-bisphosphate
ROC	Receptor-operated channels
SAR	Structure–activity relationship
SDS	Sodium dodecyl sulphate
SITS	4-Acetamido-4′-isothiocyanostilbene-2,2′-disulphonic acid
SV	Simian virus
TAL	Thick ascending limb (nephron)
TEA	Tetraethylammonium
TPA	12-*O*-Tetradecanoyl-phorbol-13-acetate
VOC	Voltage-operated channels

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Electrostatic Models for Ion Channels and Pumps

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Introduction

Most of the models used to mimic the behaviour of biological systems at a molecular level are today mechanical. For example, the explanation of the selectivity of an enzyme for a particular substrate is almost invariably that the particular shape of part of the substrate surface is accommodated by a similarly shaped cavity in the enzyme surface, often called the lock and key mechanism.

From the point of view of a physically trained scientist the great disadvantage of mechanical forces is that they are necessarily of very short range. In the example above the substrate need move a distance of only atomic dimensions before all the information contained in the shape of the enzyme cavity is lost. This is a particularly severe handicap when the surfaces of proteins in solution are known to execute thermally excited motion with a root mean square amplitude of 0.1 or 0.2 nm (McCammon & Karplus, 1983).

In contrast to mechanical forces, electrostatic forces have a very long range with the electrostatic potential of a charge falling off with distance R as $1/R$. An electrostatic enzyme–substrate recognition model would have a distribution of electric charges and electric fields at one surface of the substrate that complemented the charges and electric fields at the surface of a particular part of the enzyme so that, positioned together with a particular relative orientation, the electrostatic energy would be a minimum. Two major differences with the mechanical model would be evident, both due to the much longer range of the electrostatic forces. The first is that the sources of the electric field need not be located at the surface but could be buried in the core of the molecule and thus subject to smaller vibration amplitudes and protected from the bombardment of external molecules. The second is that even the surface charges could produce an easily recognizable and distinct field distribution at a distance from the surface, even if the surface is subject to random motion, provided that the range of the motion is small in comparison with the range of the electrostatic fields.

In fact it is likely that in Nature both electrostatic and mechanical recognition are used with electrostatic forces guiding and pre-orientating the substrate at long range (Matthew *et al.*, 1983; Getzoff *et al.*, 1983) whilst the final 'docking' of the substrate is achieved by a combination of both electrostatic and mechanical forces.

Turning now to the particular concern of this paper, namely ion channels and pumps, I am struck by the fact that although the ions, being charged, must be directly influenced by electric fields, the literature is dominated by mechanical

gating particles, mechanical selectivity filters and mechanically operated pumps. In an attempt to redress this balance I shall offer a personal view of alternative electrostatic models in the three particular areas of ion channel selectivity, ion channel gating and ion pump operation.

Ion Channel Selectivity

Many of the ion channels found in Nature are highly selective as to the ions they will allow to pass. For example, at the node of Ranvier in frog nerve fibres the sodium channel shows a preference for Na^+ over K^+ of 12:1 whilst the potassium channel exhibits a preference for K^+ over Na^+ greater than 100:1 (Edwards, 1982). Probably the most quoted model for a selectivity filter is that of Hille (1972), who postulates an annular restriction in the path of the ions of dimensions 0.3×0.5 nm lined with negatively charged groups. The selectivity of the filter depends primarily on its mechanical size and partially on the ability of the negatively charged groups to complete hydrogen bonds. The cations are supposed to travel partially hydrated so that for example 3 water molecules with the Na^+ ion fit the annulus and 4 water molecules with the K^+ ion also fit. In view of our present knowledge of the structure of ion channels, namely that the acetylcholine channel is an oligomer of five protein blocks loosely coupled with the pore defined between them (Changeux *et al.*, 1984), or that the sodium channel is most probably defined as a pore between the four largely α-helical sections of a single large protein (Noda *et al.*, 1984), it seems to me unlikely that a mechanical annulus exists with a sufficiently precise shape to distinguish between ions which differ in radius by fractions of an Ångstrom unit. This is particularly so when account is taken of the likely surface motion of amplitude 0.1 or 0.2 nm referred to above and also the relative motions of channel protein subunits.

Alternative models of the selective mechanism in ion channels are charged site models and ordered water models. Both are electrostatic and both stem from consideration of the essentially electrostatic barrier presented to a small ion by a lipid bilayer. An isolated ion of charge Q_I coulombs and radius R_I metres has an electrostatic self energy (or Born charging energy) given in joules by U_s in:

$$U_s = Q_I^2/(8\Pi\epsilon_0 R_I)$$

where $\epsilon_0 = 8.85 \times 10^{-12}$ F/m. For Na^+, U_s is 1.2×10^{-18} J/ion or 722.4 J/mol. The energy may be thought of as residing in the electric field created by the ion which extends to infinity. If, however, the ion is placed in a polarizable fluid such as water the field of the ion tends to align the dipole moments of neighbouring water molecules to point radially away from the ion. The field of the ion now partially terminates on the negative ends of the water dipoles presented to the ion and is thus much more local so that the electrostatic self energy falls drastically and the ion is said to be partially solvated. The electrical polarizability of the interior of a lipid bilayer is very small so that a bare small ion within a bilayer has a high electrostatic self energy and the probability of such an ion spontaneously leaving an aqueous environment to enter the bilayer is quite negligible. Thus the chief function of an ion channel is to provide complementary

charges or polarizable dipoles that enable the ion to traverse the lipid bilayer without having to surmount such a large energy barrier (Parsegian, 1969; Edmonds, 1980).

In charged site models much of the solvation of the ion during transit is achieved by oppositely charged sites embedded in the protein wall of the channel and the ion is supposed to traverse the channel by migration between these sites. An advantage of such models is that they are easily made selective by varying the charge $-Q_s$ and effective radius R_s of the charged site. The electrostatic energy of binding to this site for an ion of charge Q_I and radius R_I is given by U_B in:

$$U_B = -Q_s Q_I/(4\Pi\epsilon_0(R_s + R_I))$$

The difference in energy between the water solvated ion and the site bound ion varies with Q_s, Q_I, R_s and R_I, leading to the well known Eisenman (1962) series, giving the relative preferences of a given site for binding ions of different valence and radius.

The disadvantages of charged site models relate to the high electrostatic energy cost of locating the charged sites in a low dielectric constant environment and in the fact that they may not be placed close together without incurring an additional high cost in electrostatic repulsion energy. High selectivity necessitates tight binding at the charged sites and the enforced wide separation of the sites leads to large potential barriers between sites and hence to low transfer rates. In Nature highly selective channels have transfer rates as high as 10^7 ions/s.

These problems are not encountered in ordered water models (Edmonds, 1981*a*, 1984*b*) in which the ion solvation is achieved entirely by the electrically neutral water molecules within the channel. However, if the channel water behaved like bulk water there would be no ion selectivity so that it is necessary to assume that the water within the channel is structurally ordered as outlined below. There are many examples known in which water forms ordered layers several molecules thick with the structural order partially dictated by hydrogen bonding to a particular substrate, but the natural material that most closely illustrates some of the properties required by ordered water channel models are the zeolite and clay minerals. In hydrated zeolites an aluminosilicate skeleton defines approximately spherical polyhedra interconnected by tubes. The cavities and tubes contain ordered arrays of water molecules so that, for example, in hydrated zeolite A the large cages contain a distorted dodecahedron of 12 water molecules. Diffusion studies reveal (Barrer & Rees, 1960) that small ions like Na^+ and K^+ move through these structures with high mobility and moreover that only the bare ion moves and is hydrated in transit by the essentially static water molecule arrays.

The ordered water model postulates that the charge structures on the surface of the particular protein rods that define the channel impose a particular order on the water structure and thus provide a multitude of closely spaced selective ion binding sites at the centres of the various water rings in the structure. While retaining its normal tetrahedral bonding geometry, water is capable of forming two small rings, a planar pentagon and a puckered hexagon. An ion at the centre of a water ring has an electrostatic interaction with the electric dipoles of the water molecules of the ring sufficiently strong to re-orient the water dipoles to

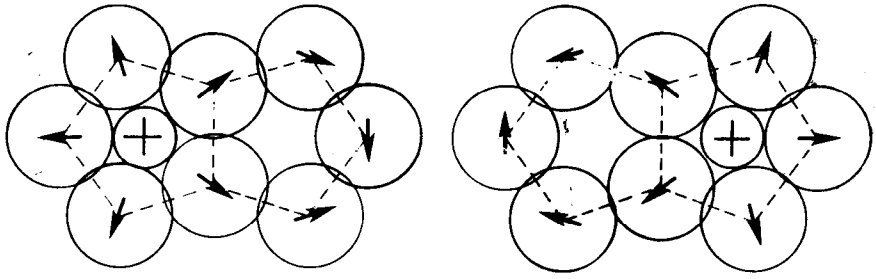

Fig. 1. *Two adjacent planar pentagons composed of water molecules*

Arrows show the orientations of the electric dipole moments of the water molecules as a Na^+ ion moves from the centre of one water ring to the centre of the adjacent water ring. Calculations show that water ring sites such as these with rotated electric dipole moments form low energy selective binding sites for small cations.

point radially away from the ion at the ring centre. Calculations which include ion–water dipole, ion-induced water dipole, ion–water quadruple, water dipole–water dipole and water quadrupole–water dipole interactions, and which include the energy cost of broken water–water hydrogen bonds, reveal that particular water rings provide especially low energy sites for particular ions with energies comparable with the fully hydrated ion (Edmonds, 1980). The planar pentagon rings favour the Na^+ ion while the puckered hexagon rings favour K^+, partially due to the almost exact match in each case of the diameter of the bare ion and the diameter of the free 'hole' in the ring centre. The ions pass through the water channel by passing between the centres of contiguous water rings as illustrated in Fig. 1, which also shows the rotation but not translation of the water molecules that is required to solvate the ion and to provide the selective water ring ion binding sites. An essential difference between the ordered water model and charged site model is that the selective ring sites may be as close together as 0.3 nm without incurring an electrostatic repulsion energy penalty, so that very high transfer rates become possible.

Channel Gating

Besides the spatial order imposed on the water lining the pore by the supporting protein rods, calculations predict that an array of water molecules within a long and thin cylindrical pore in a low dielectric constant sheet will be electrically ordered. The two states with the lowest electrostatic interaction energy retain the same spatial orientation of the water molecules but are such that the electric dipole moments of the molecules have predominantly positive or predominantly negative projections on the channel axis (Edmonds, 1979, 1984*b*). These two electrically polarized states with equal minimum electrostatic energy are sketched in Fig. 2 and below them is sketched the resultant dipolar electric potential distribution that would be measured along the channel axis. A voltage applied across the membrane will create an energy difference between the two polarized states and can thus lead to switching. For example one water structure composed entirely of pentagons is a stacked series of dodecahedrons,

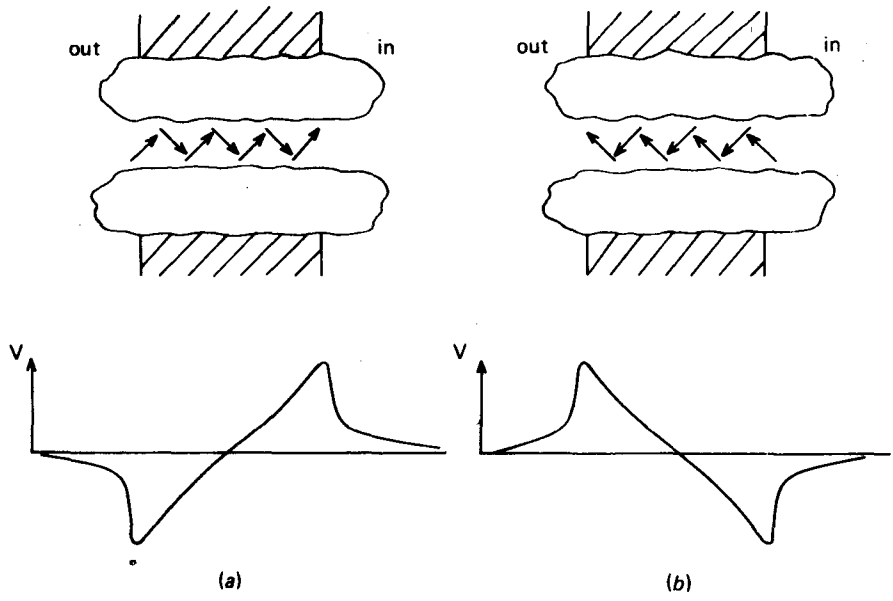

Fig. 2. Diagrammatic section of the proposed ordered water ion channel

The protein rods that define the channel are indicated and the water molecule electric dipoles are suggested by arrows predominantly pointing parallel (*a*) or antiparallel (*b*) to the inward pointing axis of the channel. Below is plotted the axial electrostatic potential distribution resulting from the composite electric dipole moment of the channel.

and such a structure with a diameter about 10 nm and a length of 48 nm has an energy difference between the two oppositely polarized states of 5×10^{-20} J or 12.4 kT when a voltage of 100 mV is applied across it (k is the Boltzmann constant and $T = 290$°C). Effective gating of the ion current through the channel is ensured by the very different current versus voltage characteristics of channels in the two polarizations.

In Fig. 3(*a*) is displayed the current versus voltage characteristic for a sodium channel using a simplified dipolar distribution (Edmonds, 1981*b*) with an external Na^+ concentration of 440 mM and an internal concentration of 50 mM. The switching voltage, at which the electrostatic energy of the water array (under the influence of the membrane voltage V_M and any fields due to neighbouring charges) passes through zero, was chosen as -25 mV as in the squid axon. In Fig. 3(*b*) is displayed as a full line the prediction using exactly the same model parameters but for a potassium channel with outside and inside potassium concentrations of 20 mM and 200 mM respectively and with a switch occurring at a membrane voltage of -50 mV, again for direct comparison with the squid axon. In both these cases the very simple model yields results that resemble those experimentally determined with no need to postulate any gating mechanism other than the switch of the axial dipole moment of the spatially fixed water array.

One particularly interesting difference between this model and the traditional mechanically blocked models is that both configurations of the channel conduct

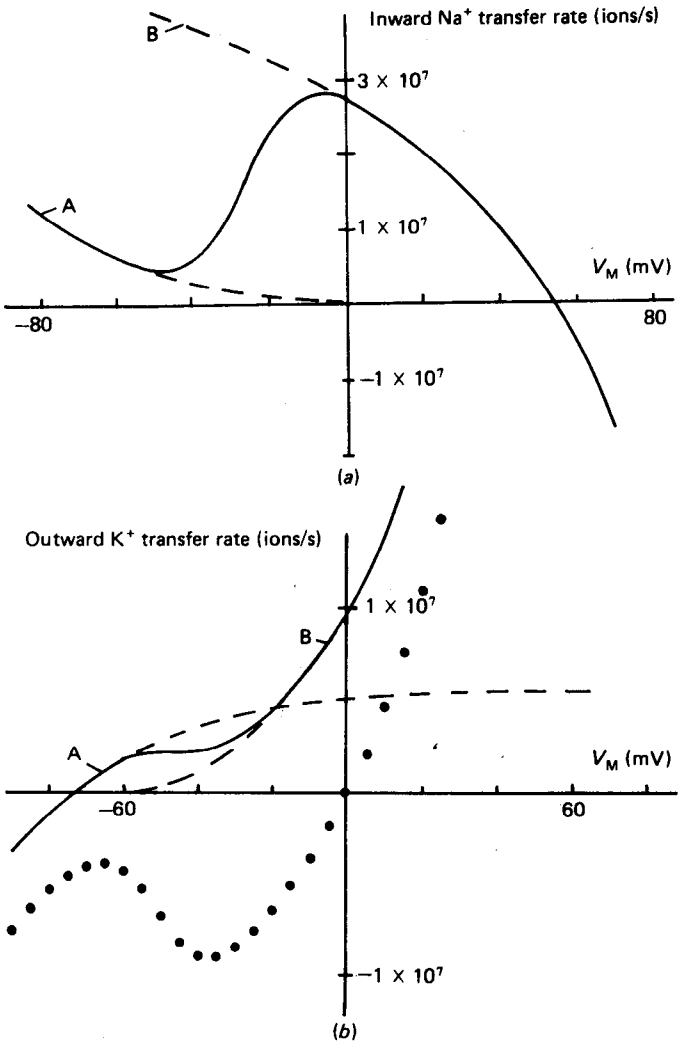

Fig. 3. *Current versus voltage characteristics for model sodium and potassium channels*

(*a*) The current versus voltage characteristic calculated for a model sodium channel with an electric dipolar structure of peak amplitude 150 mV. The external free Na^+ concentration is assumed to be 440 mM and the internal concentration is assumed to be 50 mM to mimic the sodium channel in the squid giant axon. The characteristics marked A and B correspond to the polarizations sketched in Figs. 2(*a*) and 2(*b*) respectively, with the full line giving the total characteristic corresponding to a switch between the two polarizations centred at a membrane voltage $V_M = -25$ mV. (*b*) The predicted characteristic of a potassium channel using the same model parameters but with external and internal free K^+ concentrations of 20 mM and 400 mM and a switching membrane voltage of -50 mV. The dotted line is the prediction if the external concentration is changed to 400 mM but all other parameters remain the same.

ions; it is in a high or a low conductivity state at any membrane voltage rather than being open or shut. In Fig. 3(*b*) a dotted line shows the prediction of the same simple model with K^+ concentrations both inside and out of 400 mM. Such a characteristic with a negative resistance region is observed experimentally (Segal, 1958; Moore, 1959). To obtain such a characteristic a switch is required between two configurations, both conducting. With an open or shut mechanically blocked model this requires one type of channel to shut when the membrane is polarized and another type of channel to open, with the two types of channel having similar switching voltages. With the electrostatic model the switch is merely between the two polarizations of the same type of channel. Several other phenomena observed in Nature may be explained by this simple electrically gated model (Edmonds, 1981*b*).

A second interesting feature of the model is that a multiply connected water array is probably unique in its property of being able to solvate, and thus to transmit, heavy ions such as Na^+ and K^+ and also to conduct protons by proton hopping as in ice. The model predicts that an ordered water gated ion channel should also serve as a gated proton channel. Evidence of gated proton conductivity probably through a heavy ion channel has been obtained by Thomas & Meech (1982) for a molluscan neuron.

An Electrostatic Pump

Consider a selective channel with a dipolar electrical structure such as that shown in Fig. 2(*a*). For this model any potential distribution with positive and negative lobes will suffice and the dipole moment need not switch or be due to the water dipoles. The required fixed dipolar structure could, for example, originate from the known large dipole moment (Hol *et al.*, 1978) of a neighbouring α-helical protein fragment. For definiteness of description I will assume that the channel is selective for K^+ ions. As seen in Fig. 2(*a*) there is no impediment to entry for K^+ ions from the fluid bathing the outside face but from the inner fluid they must surmount a potential barrier. In fact, calculations using the simplified linear potential show that with a peak potential of 150 mV the probability of entry is some 3000 times higher from the outside than from the inside with equal K^+ concentrations in the fluids bathing the two ends of the channel. Also by assuming equal hemispherical access volumes of radius 0.5 nm at each channel end it is easy to calculate (Edmonds, 1984*a*) that a K^+ concentration of only 0.85 mM in the outer fluid is sufficient to ensure that the channel is occupied on average half of the time. The corresponding concentration in the inner fluid is very much higher at 6.3 M.

For simplicity in the above calculations a maximum channel occupancy of 1 was assumed. In an attempt to calculate roughly the allowed occupancy of a channel, a model calculation may be performed (Edmonds, 1984*a*) in which the channel is represented as a cylinder of diameter 1 nm with high dielectric constant ϵ_1 which is embedded in a slab of material of low dielectric constant ϵ_2. Taking $\epsilon_1 = 80$ to represent the water and $\epsilon_2 = 5$ to represent the protein and lipid (Pethig, 1979) I calculate that two univalent cations on the cylinder axis have an electrostatic repulsion energy of 2.2×10^{-20} J or 5.6 kT when separated

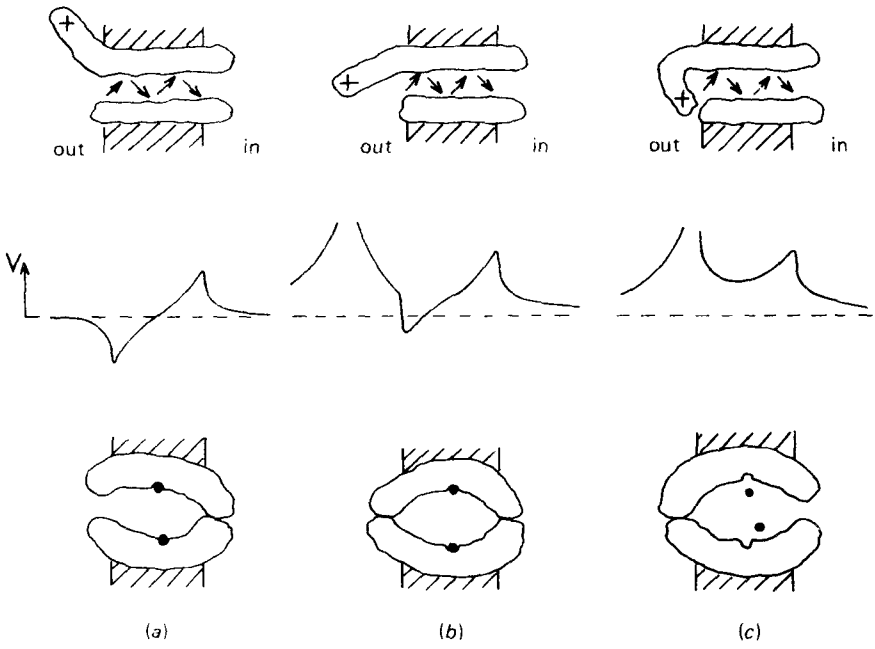

Fig. 4. *Effect of approach of positively charged group to the outer end of the ion channel*

(*a*) The dipolar channel as sketched in Fig. 2(*a*) but with a mobile positively charged group attached to the outer end of the channel support protein. The charged group is assumed sufficiently remote so that an undistorted dipolar axial potential distribution is obtained. Below is sketched an approximately equivalent mechanical model showing high affinity binding sites exposed to the outer fluid only. (*b*) The effect of a closer approach of the charged group to the channel end showing the axial electrostatic potential distribution which is now a composite of that the channel dipoles and the charged group. The potential now shows considerable barriers to ion flow at both ends of the channel, and the mechanical model also shows occuluded states. (*c*) Closer proximity of the charged group results in the tipping of the composite potential distribution with the consequent expulsion of cations within the channel to the inside. The mechanical model shows low affinity sites now exposed only to the inside.

by 2 nm. Thus a 5 nm long channel could simultaneously contain 2 or perhaps 3 univalent ions but is highly unlikely to contain more.

Let us now consider the effect produced by a positively charged group approaching the outer end of the channel. In Fig. 4(*a*) is shown the channel in the absence of the charge with its undistorted dipolar potential distribution. In the lower part of Fig. 4(*a*) is the roughly analagous state of the traditional mechanical model of a membrane pump due to Jardetski (1966). In each case the pump exposes high affinity binding sites to K^+ ions from the outer fluid whilst effectively barring entry for K^+ ions from the inner fluid. In Fig. 4(*b*) the positively charged group approaches the outer end of the dipolar channel. The electrostatic potential experienced by ions within the channel is then a simple superposition of the dipolar channel potential and that from the charged group as sketched in the Figure. The ions within the channel are now occluded with little probability of leaving the channel from either end. Even closer approach of the charged group as shown in Fig. 4(*c*) results in a tipping of the whole

potential distribution towards the inner end of the channel. Calculation confirms (Edmonds, 1984*a*) that the dwell time of cations within the channel is much reduced and the probability of exit from the inner end tends to unity. Again the roughly equivalent state of the mechanical model is shown below with low affinity sites having access only to the inside. Unlike the mechanical model the changes in access and affinity are readily explained; they require no change either electrical or mechanical in the channel or its support protein but only the passive superposition of two electrostatic potentials.

It is clear from the description above that any mechanism that brought about an alternation between the states shown in Figs. 4(*a*) and 4(*c*) would result in the unidirectional pumping of 2 or 3 K^+ ions each cycle from the outer to the inner fluid even against a large adverse voltage or concentration gradient. An externally applied oscillating membrane voltage would substitute for the mobile charged group and would also result in ion pumping. This is of interest because such an applied oscillating membrane voltage has been shown (Serpersu & Tsong, 1983) to result in an ouabain sensitive Rb^+ ion uptake in human erythrocytes and Rb^+ is known to substitute for K^+ in the Na^+, K^+-ATPase pump of these cells.

An energy input would clearly be required to activate the pump, as is seen from Fig. 4. If the positively charged group is spontaneously attracted to the negatively charged outer end of the channel, even while occupied, then an external agency would be required to remove the charged group (Fig. 4*c* to Fig. 4*a*) after the cations within the channel have been expelled to the inner fluid. In a more complete model this action would be powered by the binding of ATP. A model of Na^+,K^+-ATPase may be constructed by postulating two such dipolar channels in close proximity with a sodium selective channel polarized outward and a potassium channel polarized inward as in Fig. 4(*a*). Many properties of real Na^+/K^+ exchange pumps may be simulated by these means (Edmonds, 1984*a*). I will not develop this further here but will mention one interesting aspect of the simulation. Like the electrostatic gated ion channel model the electrostatic pump channel is never fully open or shut. In the configuration of Fig. 4(*a*) the probability of entry from the inside is small but not zero. This feature becomes important when using the model to simulate well-established but non-physiological modes of action in which the pump is forced to reverse.

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