## CARRIERS AND CHANNELS

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# ION SELECTIVITY OF CARRIERS AND CHANNELS

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Participants in the discussion of carriers and channels expressed a great deal of interest in how one defines such entities. Admittedly, it is not easy. In the case of channels in the excitable membrane, we think that these really are channels since large electric currents can be passed without saturation and we know of no carriers that exhibit this behavior. We tend to think of active transport phenomena as effected by carriers mainly because rates are slow. One way of understanding something about ion transport mechanisms is to look at the manner in which ion selectivity is maintained.

There are basically two mechanisms by which ion selectivity in biological systems can be obtained: a fixed charge system such as that exhibited by certain glasses (as worked out in great detail by Eisenman [1962]) and a solvation replacement system (as suggested by Mullins [1959, 1968]). The first method depends on a strong binding by the selected ion to a fixed charge site and hence it is not a method that can be used for selectivity when large electric currents must pass the membrane (such as in the operation of a Na channel in an excitable membrane).

The second method requires that an ion give up its hydration in aqueous solution only if this can be substituted by an equally close-fitting layer of solvation provided by an organic molecule that contains highly polarizable atoms. An ion that is too large will not enter a cage or pore; nor will an ion that is too small, because the interaction of such an ion with the substitute solvation is not as strong as the ion's interaction with aqueous hydration. Of course, these considerations apply only to carriers or channels that are rather rigid, not easily deformable structures. For flexible structures one can expect that there will be little or no cation selectivity since the structure involved will adapt itself to the size of the ion it encounters and hence it will not be an ion selective system.

Biological transport systems involving ion selectivity can be divided into three categories. In one type the selectivity of Na to K (for example) is much greater than 100:1 (Na efflux energized by the ATP-dependent Na pump), in another the Na/K selectivity is of the order of 10:1 (the Na channel of the axon membrane), and finally there are systems with very low selectivity (the acetylcholine receptor of the muscle end-plate membrane).

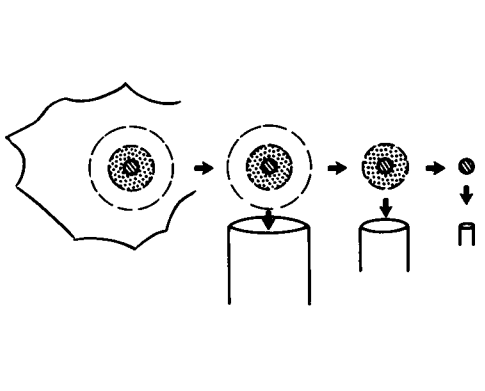

FIGURE 1

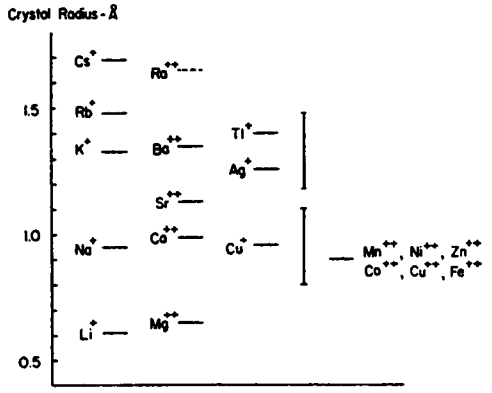


FIGURE 2

FIGURE 1 An ion in bulk water (left) is capable of being separated from infinite hydration in three ways: (*a*) as a double layer of water molecules, (*b*) with a single layer of hydration, and (*c*) as a totally dehydrated ion. An ion of each size is capable of fitting an appropriately sized channel (see below each ion size) but the ion selectivity *is* high only for the dehydrated ion.

FIGURE 2 Crystal radii for the alkali cations, the alkali earth cations, some monovalent heavy metal cations, and some divalent heavy metal cations are shown. For a Gaussian distribution based on Na, and with K 1/100 the ordinate for Na (or for one based on K with Na 1/100), the vertical bars show ±1 SD on a Na- or a K-sized distribution.

The proposition to be developed here is that (*a*) high ion selectivity necessarily involves the total dehydration of an ion and its enclosure in a very close fitting structure which effectively solvates the ion, (*b*) moderate ion selectivity means that the ion involved is partially hydrated but with not more than a single layer of water molecules between the ion and the structure providing solvation, and (*c*) a system with low or negligible ion selectivity will take an ion into a structure with more than one layer of aqueous hydration. These arrangements are shown in Fig. 1. A consequence of these varying hydration arrangements is that systems with low or negligible ion selectivity can carry very large ionic currents because of the low energy barrier for the detachment of an ion from aqueous hydration and its insertion into a channel. By contrast, high ionic selectivity (greater than 100:1) involves a very high energy barrier and cannot be expected to be compatible with a high velocity of ion transfer (or with large ionic currents).

*Ion Size.* An examination of tables of ion crystal radii shows that one can divide the alkali metal cations into small sized ions (Na, Li) and large sized ions (K, Rb, Cs). The alkaline earth cations can be similarly divided and the crystal radii of Ca and Na are virtually identical, as are Ba and K. Fig. 2 shows these crystal radii along with two bars representing one standard deviation of crystal radius size. From this figure it is evident that one can include virtually all ions of physiological interest in one or the other of these two distributions—Na-sized and K-sized.

If one suggests that ions go through membranes in a singly hydrated state, one would predict that divalent cations ought to go through the channels of excitable membranes

depending on whether they are similar in size to Na or to K. In the case of the Na channel there is very clear evidence that $Ca^{++}$ go through the Na channels and carry a very small fraction of the depolarizing current (Hodgkin and Keynes, 1957; Meves and Vogel, 1973). Clearly this is a specific action since the extra Ca influx with stimulation is abolished by tetrodotoxin (Baker et al., 1971). Indeed, if the membrane resistance of a squid axon is made quite high by internal perfusion with CsF, and the $[Ca]_o$ is increased, a slow action potential can be generated in the absence of any cation in seawater other than Ca (Tasaki et al., 1968). Although Ca actually penetrates substantially better than hydration calculations would indicate, this finding does not present a major difficulty since the kinetic details by which even $Na^+$ surmount the potential energy barrier are unknown.

Barium ion is an analog of K; hence one might expect this ion to exhibit the same sort of behavior in the K channel as that exhibited by Ca in the Na channel. Owing to the peculiar structure of the K channel (to be discussed later), poisoning and competition tend to be favored rather than transport. Nevertheless, it has been possible to show in muscle (Mullins, 1959) that there is an increase in Ba efflux associated with the action potential. A further prediction based on a crystal size analysis was that $Tl^+$ would be a penetrating cation. Measurements showed that Tl depolarized frog muscle membrane as well as $K^+$ and that its tracer flux was larger than $K^+$ when differences in concentration were allowed for (Mullins and Moore, 1960). Further studies showed that $Tl^+$ was the most permeable cation in squid axon (Hagiwara et al., 1972) and that it went through the K channels of the nodal membrane with a permeability superior to that of $K^+$ (Hille, 1973).

*The State of Ions in Aqueous Solution.* Measurements of the limiting ion conductances in aqueous solution give highly confusing information about the nature of an ion that may be penetrating a biological membrane. The conductances show that Cs > Rb > K $\gg$ Na $\gg$ Li, while measurements for K channels in nerve show that K $\gg$ Cs or Na, and for the Na channel Na = Li $\gg$ K. One can argue that what aqueous conductances really measure is the extent to which water has sites of appropriate size for an ion to occupy.

Hydration energies for the alkali cations in aqueous solution are very large (Na, 100 and K, 75 kcal/mol) so that any appreciable concentration of totally dehydrated ions cannot be expected. A simple calculation shows that one-half of the hydration energy is involved in the binding of the first complete shell of water molecules around an ion (Mullins, 1956) and that about 75% of the hydration energy is accounted for by two complete shells of hydration. Steric considerations suggest that a hydration shell for Na or K consists of six water molecules so that for Na, the binding energy per water molecule in the first hydration shell is about 8 kcal (50 kcal hydration energy in the first shell/6). Thus energy considerations suggest that a first hydration shell has some physical reality. A second hydration shell has both a large number of water molecules and only half the hydration energy; hence the binding of any individual water molecule only slightly exceeds thermal energy and the shell is a much more diffuse structure.

*Special Hydration Configurations.* It has been suggested that since excitable membranes depend primarily on rapid *cation* movement for their bioelectric properties, fixed negative charges are an important consideration in the structure of such membranes. This is not necessarily the case because hydrated cations have a "hydrogen out" orientation of water molecules that will interact with polarizable oxygens in a carrier or pore, while anions have an "oxygen out" orientation that will interact with a different sort of lining of a carrier or pore.

Lithium and other small cations (crystal radius 0.60 Å) are a special case with respect to hydration configuration. A six-water hydration configuration does not fit the ion closely but it is not possible to design a water shell with a smaller number of water molecules which *does* fit such an ion. The presumption is that if Li penetrates with a single water shell, its structure will be that of a six-water shell. Since Mg has virtually the same crystal radius as Li, one might expect singly hydrated Mg to behave as does Ca and carry some current through the Na channel. Since this clearly is not the case one infers that the arrangement proposed for Li may not be stable in the presence of a divalent cation.

A related problem concerns polyatomic organic cations of a variety of sorts. Such cations are important because they have been shown to penetrate the Na and K channels of excitable membranes. In the first place, there must be somewhat more uncertainty regarding the size of organic cations because information about crystal radius is seldom available and one is forced to measure from atomic models. In addition, charge is frequently not on the central atom of a polyatomic ion, but shared between several atoms of the ion. Finally, some substituents in a polyatomic ion are often nonpolarizable $CH_3$ or $C_2H_5$ groups. All of these considerations dictate considerable caution in trying to relate size of organic cations to sizes of alkali metal cations. Perhaps the simplest polyatomic cation is $NH_4^+$; the crystal radius of this cation is known and it is a cation that passes through the Na and K channels of excitable membranes with about equal ease. For this ion it seems reasonable to assume that the single water shell hydration configuration starts from the surface of the central nitrogen atom and hence one uses not the crystal radius but the radius of N as the start of the water shell. The methylation of $NH_3$ to form methyl amine has two effects—first it destroys the integrity of the water shell surrounding $NH_4$ and second it results in a loss of shielding of the central charge in the region of the $CH_3$ group. The effect to be expected is that of giving the ion a highly asymmetric hydration shell and making it more difficult to remove the ion to a channel or carrier. Further speculation about the hydration configuration of organic cations is unwarranted, except to note that for structures such as tetraethylammonium, the charge is so remote from access to water and the ion so large, that it is a reasonable approximation to regard the ion as unhydrated.

*Systems of High Ion Selectivity.* When a squid axon is deprived of ATP and other substrates by dialysis of the axoplasm, Na efflux is at a level of from 1 to 1.5 $pmol/cm^2 \cdot s$ and this is quantitatively accounted for by flux ratio considerations (Brinley and Mullins, 1968). If ATP is then added to the dialyzed fiber, Na efflux

increases to normal levels (assuming that the axon is bathed externally in normal seawater [Brinley and Mullins, 1968]). If the normal membrane potential of such an axon (−60 mV) is raised to −75 mV or more, Na efflux is unaffected (Brinley and Mullins, 1974). If the same experiment is repeated using labeled K rather than Na, addition of ATP produces no effect on K efflux as long as the membrane potential is kept at −75 mV or more (to keep K permeability constant). One concludes that within the error of measurement, the Na pump mechanism discriminates absolutely between Na and K and that the selectivity of the Na pump in favor of Na over K must be at least 100:1. Similar conclusions can be reached by studying isolated, purified membrane ATPase.

If Na efflux is measured as outlined above with $Na_i$ fixed at 10 mM and then Li is introduced into the fiber at a concentration of 100 mM, there is no effect of Li on the Na efflux; again the conclusion is that the selectivity of the Na pump Na/Li is at least 100:1 in favor of Na. The information with respect to Li is important because, as will be shown later, the Na channel of the excitable membrane is unable to discriminate between Na and Li. The question then arises: how is it possible to have a selectivity mechanism that in one case clearly discriminates between two ions and in the other case does not. The answer would appear to lie in the fact that the crystal radius of Li is 0.65 Å while that for Na is 0.95 Å. As has been indicated above, for a single layer of hydration around these ions the water cage cannot shrink much below Na size so that with a single hydration shell, both Na and Li have the same size. One can also note that the partial molal volumes of NaCl and LiCl in aqueous solution are the same.

A second system that has been studied in enough detail to yield information regarding ion specificity is the Na/Ca exchange system (Brinley et al., 1975). In the squid axon, this system allows the coupling of Na influx to Ca efflux and in effect is a mechanism for the maintenance of a low $Ca_i$. This system functions in the absence of substrates such as ATP and under these conditions, if $Ca_i$ is made 0.05 $\mu$M, an easily measurable Ca efflux is obtained. If internal Mg is then increased from 0 to 5,000 $\mu$M, there is no effect on Ca efflux even though the ions are similar chemically and even though a singly hydrated Mg (by analogy with Li) ought to closely resemble Ca. Again, the conclusion would appear to be that Mg and Ca behave differently because the ions are dehydrated and the crystal radii (0.6 and 0.9 Å, respectively) are different.

The conclusion from studies with both the Na pump and the Ca pump in squid axon would therefore be that the high specificity exhibited by both these transport systems exists because the ion to be transported is totally dehydrated before its incorporation into the transport system and that total dehydration is a necessary requirement for high ion specificity.[1]

*Systems of Moderate Ion Selectivity.* The system of moderate ion selectivity

[1] Ion charge must be involved in the discrimination between the Ca and the Na/K pumps in the squid axon because it is not possible to measure a Na efflux through the Ca pump even when $[Na]_i$ is $10^5$ that of $[Ca]_i$.

that has been most extensively studied is that of the Na channel of the excitable membrane of both squid axons and the frog nodal membrane. The results with the two species are so similar that they can be conveniently lumped together.

In the first place, the notion that totally unhydrated Na might penetrate through the Na channels would appear to be clearly ruled out by the finding that the large organic cation hydroxylamine ($NH_3OH^+$) penetrates about as well as Na. A partially hydrated ion of some unknown configuration would seem to be indicated and a suggestion has been made that perhaps a Na with a single molecule of water would be compatible with the facts (Hille, 1971). Aside from its energetic improbability, and energy considerations will be crucial to arriving at an exact configuration for the hydration shell of penetrating entities, there is simply not enough information available to decide on a specific arrangement of water molecules around a Na ion. Therefore my discussion will be limited to showing that a Na with six water molecules in a single hydration shell is compatible with the experimental information presently available.

Both the size of the Na channel and the size of the hydration shell around the ion will be subject to thermal fluctuations; in the treatment that follows it will be convenient to refer this size fluctuation to some kind of standard deviation around a mean channel size and once this is done, it should prove in principle possible to account quantitatively for the Na channel ion selectivity.

The assumptions in the treatment that follows are: (*a*) that Li is the most permeant ion in the Na channel, (*b*) that Li and all other ions penetrate the Na channel in a hydrated configuration, and (*c*) that in order to penetrate, a hydrated ion must have a "goodness of fit" that is ±0.05 Å. The distribution of pore sizes shown in Fig. 3 has

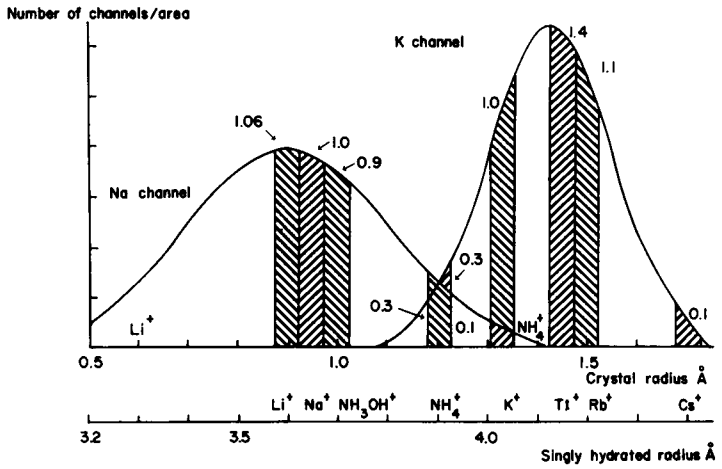


FIGURE 3 This is a plot of the instantaneous channel size distribution for Na and K channels. Curves were constructed by setting $Li^+$ at the maximum of the Na channel distribution, and $Tl^+$ at the maximum for the $K^+$ channels. Both $P_K/P_{Na}$ (Na channels) and $P_{Cs}/P_K$ (K channels) were set as 0.1. Ion size on a crystal radius basis is shown on the upper abscissa where Li has a very different size from Na, while there is little difference in the singly hydrated radius (shown as the lower abscissa).

TABLE I

SELECTIVITY OF Na AND K CHANNELS AND ACh RECEPTORS

| | No. per $\mu m^2$ | Conductance per channel $\times 10^{-11}$ | $P_{Na}/P_{Li}$ | $P_{Na}/P_K$ | $P_K/P_{Cs}$ |
|---|---|---|---|---|---|
| | | *mho* | | | |
| Na channels | 100 | 1 | 1.0 | 0.1 | |
| K channels | 100 | 1 | | | 10 |
| ACh receptors | | 10 | 1.0 | 1.0 | |

been drawn with these assumptions in mind and with the permeability of K through the Na channel arbitrarily set at 0.1 that of Na. The assumption of an arbitrary "goodness of fit" is not a very critical one as it can be increased by 2 or made 1/2 with little or no effect on the ion selectivity ratios. The main point of Fig. 3 is to show that a rather simple treatment suffices reasonably well to account for the ion selectivity that is actually observed in a Na channel.

A second system with a moderate ion selectivity is the K channel of the excitable membrane. Fig. 3 also shows a size distribution for this system constructed with the following assumptions: (*a*) that $Tl^+$ is the most permeant ion in this channel, (*b*) that the ratio of $P_K/P_{Cs}$ is 10:1, and (*c*) that the number of K channels is equal to the number of Na channels (see Table I). Again the agreement between experimentally measured ion selectivity and that calculated from this curve is reasonable. A discussion of ion selectivity in the K channel cannot be dissociated from some properties of this channel that make it different from the Na channel. The K channel can be easily poisoned by a variety of cations both inside and outside the membrane, and this fact makes selectivity measurements somewhat more complicated to carry out. For example, $P_{Cs}$ judged from voltage clamp data is near zero while $P_{Cs}$ measured with tracers is about 0.1 $P_K$.

The structure of a K channel has been rather convincingly shown to include a site at the inside capable of being occupied by a variety of cations such as Na, Cs, tetraethylammonium (TEA), and analogs of TEA with a long paraffin chain (Armstrong, 1966). When such a site is occupied, the K channel is effectively blocked for K currents directed from inside out, but it is not blocked for currents moving in the opposite direction, suggesting that TEA is not strongly bound. This site must be large enough to accommodate a cation of the size of TEA, as well as smaller cations such as Cs and Na. All these requirements can be met if such a site is large enough to accommodate Na or Cs with *two* complete shells of hydration because, as will be suggested below, ions with two such shells cannot be distinguished on the basis of the size of the crystal radius of the ion. That is, the hydration shell is so fuzzy that differences in ion size are lost in the thermal dispersion of the water shells. At any rate, the suggested arrangement of an inner site allows ions of a variety of sorts to enter the site. However, only an ion close to "K size" will pass through the channel because such passage re-

TABLE II
SELECTIVITY OF Na AND K PUMPS

| Pump flux | Na pump | K pump |
|---|---|---|
| Na/Li | >100 | |
| Na/K | >100 | |
| Li/K | | 0.05 |
| Rb/K | | 0.50 |
| Tl/K | | 1.50 |
| Cs/K | | 0.10 |

quires the shedding of a layer of hydration so that the ion goes from two shells to one shell of water. In other words, all ions entering the K channel from inside the membrane do so by a two-stage process that involves the following sequence: $X^+$ (solution, infinite hydration; $X^+$ (two-shell hydration; $X^+$ (one-shell hydration); $X^+$ (outside, infinite hydration if $X^+$ is of penetrating size).

The outside of the K channel in the squid axon also has a site that does not admit TEA but does admit $Cs^+$ so that K channel blocking with $Cs^+$ is possible from both sides. In sharp contrast, the Na channel does not exhibit any phenomena at all comparable to the TEA effect in the K channel, which must mean that the Na channel has sites along its length that are all very similar. This point is an important one because it is sometimes suggested that selectivity is effected at a single site and that after an ion passes the site where selectivity is determined, its further movement along a channel is nonspecific. This seems most unlikely because the channel radius in the selective region would have to be 3.67 Å (the size of a singly hydrated Na) and would have to be over 6 Å in the rest of the channel. Such a channel could accumulate other ions such as K in its nonselective region and should then exhibit the TEA-like effect shown only by K channels.

A third system of moderate ion selectivity is the ATP-dependent pump that moves K or other monovalent cations inward in a manner that is presumably coupled to outward Na movement. Unlike the Na pump whose specificity for Na is virtually absolute, the K pump will move a variety of alkali cations, as well as $Tl^+$. The *specificity* of this system actually resembles that of the K channel in the excitable membrane although its biochemical properties (sensitivity to ouabain and ATP) are of course quite different. The properties of the Na and K pumps are shown in Table II, and the conclusion to be drawn is that the K pump behaves as if it moves a singly hydrated ion inward (in contrast with the Na pump moving an unhydrated Na outward).

*Systems of Poor Ion Selectivity.* It has been known for some time that the ACh sensitive channels of the motor endplate membrane are unable to discriminate between Na and K. It has also been known that these channels will readily pass ions such as $CH_3NH_3$ and other substituted amines (Furukawa and Furukawa, 1959) while in the Na channel of the excitable membrane such methyl substituted amines are absolutely impermeable. Such a differing behavior for the same ionic species in two separate channel systems is most easily explained if the degree of hydration of the ion

is greater for the less selective channel. Since, the Na channel must take ions with a hydration shell, it seems necessary to suppose that the acetylcholine channel takes ions with two or more shells of hydration. Since a double hydration shell is perturbed enough thermally so that it has little or no precise shape, a double shell is sufficient to confer a lack of ion specificity.

From energetic considerations it ought to be considerably easier to detach an ion with a double hydration shell than one with a single shell. This should mean that the ion conductance per channel ought to be substantially greater for the acetylcholine receptor than for the Na channel. Estimates of numbers of Na and K channels per unit area, and hence the conductance per channel, are subject to substantial errors and perhaps only an order of magnitude estimate is possible at this time. However, as the data of Table I show, one can estimate a 10-fold greater channel conductance for the ACh receptor.

*Gating vs. Ion Selectivity.* In most model systems for a Na channel, "gating" and "selectivity" are separate functions. One is provided by an accessory known as a selectivity filter, the other with a door or "gate," most commonly at the inside of the Na channel. A difficulty with such an arrangement is that if Na is selected for by a dielectric or solvation replacement model at the outside of a Na channel, the ion still needs this solvation along the entire length of the channel, or, the channel needs to become very large so that it passes all sorts of ions without any selectivity. While this latter alternative is quite possible, it in turn means that ions inside the nerve fiber will have free access to the channel. Since these are principally K, and K by definition does not readily pass the selectivity filter on the outside of the membrane, one would expect Na channels to exhibit a TEA-like effect caused by $K^+$ in the nonselective part of the Na channel. One might think that with a gate at the inside of the Na channel, $K^+$ would be kept out. This hardly helps, however, as upon opening the gate with a

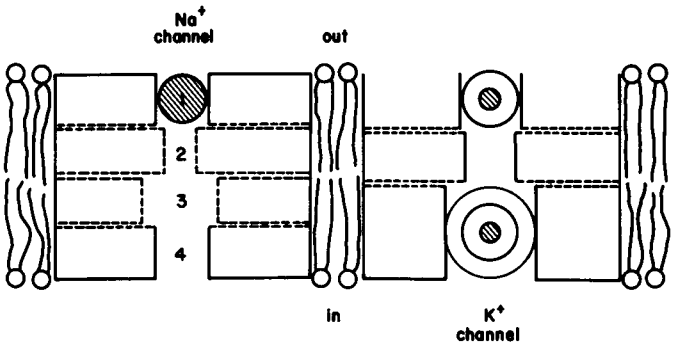


FIGURE 4 Arrangements in both Na and K channels that may be connected both with ion selectivity and with gating. In the Na channel, two fixed sites at the outside and inside of the membrane keep the channel interior largely free of ions other than Na. The channel is open only when interior sites are at "Na size." In the K channel, an interior site large enough to accept doubly hydrated ions is necessary to account for the inhibition of outward K currents by ions as diverse as $Cs^+$, $Na^+$, and $TEA^+$.

highly depolarizing potential many more $K^+$ would enter the channel than would Na and one would find K highly inhibitory to Na current. Experimentally this is not observed and in fact, a high $K_i$ makes Na channels more selective for Na (Cahalan and Begenisich, 1975).

If a Na channel is as long as the lipid bilayer is thick, then a singly hydrated $Na^+$ of 7.4 Å diameter occupies about 1/4 of the channel length. One could imagine, therefore, a Na channel composed of 4 independent subunits each of which would have to have its core at Na size for the complete Na channel to be open, while if any one of the subunits had a different size for its core, the whole channel would be closed. Gating is therefore possible by the simple expedient of making the selectivity of a unit of the channel length unfavorable for Na. This arrangement, along with one for the K channel, is shown in Fig. 4. The number of possible ways of arranging gating in the model is obviously very great, but it is worth pointing out that the outermost and innermost segments of a channel could be voltage independent and fixed at Na size, while gating could be carried out by size changes of the two central segments of the Na channel.

The author is indebted to Doctors P. De Weer, L. Goldman, H. Meves, and R. Sjodin for valuable comments in connection with this presentation.

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