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The Hydration of Sodium Ions Crossing the Nerve Membrane

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ABSTRACT The sodium channel of the excitability mechanism in nerve membranes is about as permeable to hydroxylamine and hydrazine cations as it is to sodium ions. It is impermeable to methylamine cations. This selectivity is explained by supposing that an oxygen group in the channel must receive a hydrogen bond from the permeating cation at the same time as the cation lies against another negatively charged oxygen acid. If these conditions are not satisfied the cation cannot permeate. Sodium ions can satisfy this hydrogen-bonding requirement if they have a water of hydration. The $H_2O \cdot Na$ complex also has almost the same dimensions as the hydroxylamine and hydrazine cations. This hydrated ion is probably part of the critical complex between sodium ions and the selectivity mechanism of the sodium channel.

Many biological processes distinguish between sodium and potassium ions. A first step in understanding the origin of ionic selectivity is to determine the state of the alkali ion in the active complex. Is the ion hydrated, more or less as it is in solution, is it dehydrated and surrounded by polar groups provided by the biological system, or is it in some intermediate form?

The surface membranes of nerve fibers contain sodium-preferring sodium channels and potassium-preferring potassium channels. During the propagation of an impulse, the early opening of sodium channels and the later opening of potassium channels gives rise to an early influx of sodium ions and a later efflux of potassium ions. These ionic movements explain propagated action potentials (1). Although sodium is the normal substrate of the sodium channels, lithium and a number of organic cations also permeate well enough to support action potentials (2–8). The degree of hydration of sodium ions in the sodium channels is still in question.

This paper is a preliminary account of experiments which suggest that sodium ions pass through sodium channels with at least one water molecule. The argument depends on finding two organic cations which resemble sodium ions complexed with one water molecule and which pass through open sodium channels nearly as easily as sodium ions do. The results are part of a larger experimental study of the permeability of sodium channels to 25 different cations to be reported elsewhere. Potassium channels have not yet been studied in the same way.

MATERIALS AND METHODS

Electrical measurements

The relative permeability to sodium substitutes was calculated from the changes in reversal potential for ionic currents in the sodium channel when the external sodium ion was completely replaced by a sodium substitute. Most of the procedure has been described (9). Single myelinated nerve fibers from the frog *Rana pipiens* were studied at 5°C. The ionic currents across the membrane of one node of Ranvier were measured by the voltage clamp method of Dodge and Frankenhaeuser (10) using new and more stable solid-state circuits. The membrane current and voltage were recorded digitally on-line by a Raytheon PB 440 computer taking a 9-bit current and voltage sample every 20 μsec. The capacity and leakage current were removed from the total current by subtracting an appropriately scaled response to a hyperpolarizing voltage pulse. The voltage clamp figures in this paper were drawn directly by the computer after subtracting capacity and leakage currents. Outward current is up.

The nodal membrane was clamped at a holding potential of −80 mV (inside minus outside). Test pulses spanned the range from −72.5 to +77.5 mV in 7.5-mV steps. Each test pulse was preceded by a 50 msec hyperpolarization to −125 mV to remove any resting inactivation of sodium channels. Current-voltage diagrams were drawn from the peak sodium or sodium-substitute currents after leakage and capacity currents were subtracted. The reversal potential of the early currents was taken as the intersection with the zero-current axis of a smooth curve drawn through the current-voltage plot. As is described later the reversal potential was used to calculate the permeability of a test ion relative to sodium ions. The amount of "attenuation artefact" (10, 11) was measured by replacing $^7/_8$ of the sodium in Ringer's solution with impermeant tetraethylammonium cation. The reduction of the reversal potential for sodium current with reduction of sodium concentration was 12% less than the value expected from the Nernst equation, indicating that only 88% of the actual membrane potential changes were recorded at the oscilloscope. Except where noted, the potentials reported here are uncorrected for this attenuation.

Solutions

The "normal" Ringer's solution contained: 110 mM NaCl, 6 mM tetraethylammonium Br, 2 mM $CaCl_2$, 1 mM Tris·Cl buffer, pH 7.4. Terraethylammonium ion was included to block potassium channels and thereby improve the resolution for the currents passing through sodium channels (9). Potassium was left out because sodium channels are slightly permeable to it. Isotonic tetraethylammonium, Ca, and Tris solutions were all shown to give no detectable inward current through sodium channels.

Methylamine Ringer's was identical to the normal Ringer's but with the NaCl entirely replaced by methylamine·HCl (pK_a = 10.6). Hydroxylamine Ringer's had 100 mM hydroxylamine·HCl (pK_a = 6.0), 45 mM tetramethylammonium

hydroxide, 2 mM $CaCl_2$, 6 mM tetraethylammonium Br, and a pH of 5.84. The final concentration of hydroxylamine cation was 55 mM. Hydrazine Ringer's had 110 mM hydrazine·HCl (pK_a = 8.1), 2 mM tetramethylammonium hydroxide, 2 mM $CaCl_2$, 6 mM tetraethylammonium Br, and a pH of 5.99. The final concentration of hydrazine cation was 108 mM. Atomic absorption spectroscopy revealed less than 0.3 mM sodium in the hydrazine and hydroxylamine solutions. The measured osmolalities of the four Ringer's solutions agreed within 5% of each other.

RESULTS

Fig. 1 shows the currents through the sodium channel in an experiment with sodium, hydrazine, and hydroxylamine Ringer's solutions. In sodium Ringer's there is a transient net inward current for test pulses from −50 to +40 mV and a transient net outward current for test pulses to +55 and +70 mV. The currents increase and then decrease during the first milliseconds of each test pulse as sodium channels open and close. As is now well understood, the inward currents are the inward movements of sodium ions (1, 11) and the outward currents at the highest potentials are the outward movements of sodium ions with some contribution from potassium ions (4, 5).

In hydrazine and hydroxylamine Ringer's solutions, there also are inward currents which increase and decrease in a few milliseconds. These must be inward movements of the organic cations through sodium channels. The inward currents are somewhat smaller than the normal sodium currents and become outward at a smaller depolarization. In five experiments with hydrazine the reversal potential averaged 11.4 mV lower than in sodium Ringer's, and in 4 experiments with hydroxylamine, 18.0 mV lower.

On the assumption that the selectivity mechanism of the sodium channel does not change when sodium ions are removed, the relative permeabilities to sodium, hydrazine, and hydroxylamine can be calculated. The Goldman equation (12, 13) relates the reversal potential (zero current potential) to ionic concentrations and permeabilities when there is a constant field across the channel or under various other conditions. It can be used to define the measure of relative permeability (5, 8). According to the Goldman equation, when the external solutions contain only one permeant species, as in these experiments, the change in reversal potential with a

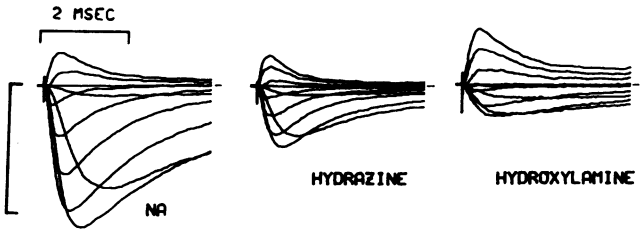

FIG. 1. The family of voltage clamp currents of a node of Ranvier bathed in three different Ringer's solutions. The ten clamp voltages are spaced at 15-mV intervals spanning the range from −65 to +70 mV. The current calibration represents 20 nA for sodium and hydrazine Ringer's and 10 nA for hydroxylamine Ringer's. Leakage and capacity currents have been subtracted.

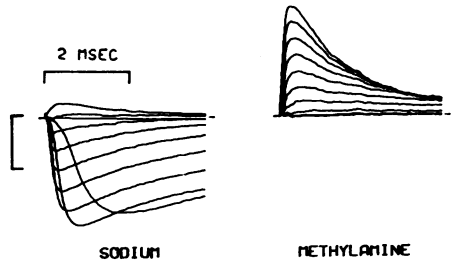

FIG. 2. The family of voltage clamp currents of a node of Ranvier bathed in sodium and methylamine Ringer's solutions. Same conditions as Fig. 1. The current calibration is 20 nA for sodium Ringer's and 4 nA for methylamine Ringer's.

change in external solution is

$$\Delta E_{reversal} = 2.303 \frac{RT}{F} \log \frac{P_S[S]}{P_{Na}[Na]} \quad (1)$$

where 2.303 RT/F is 55.2 mV at 5°C, P is permeability, S is the sodium substitute, and concentrations refer to the permeant cations in the two external solutions compared. After correction for measured junction potentials and for the attenuation artefact, the changes in reversal potential for hydrazine and hydroxylamine Ringer's are −12.6 and −19.5 mV. From Eq. (1) the permeability to hydrazine cations is therefore 0.59 of the permeability to sodium ions and the permeability to hydroxylamine cations is 0.90 of the permeability to sodium.

Re-examination of the relative amplitudes of the currents in Fig. 1 might suggest that the permeabilities to hydrazine and hydroxylamine are somehow overestimated in the preceding calculation. There are several reasons for this impression. First, only a fraction of the hydroxylamine is in the cationic form. Second, the pH of the two solutions is so low that 10–15% of the sodium channels are blocked by protons (14); and third, the substitutes (especially the unionized forms) exert some direct pharmacological (anesthetic) effect which further reduces the number of active channels. It is because the reversal potential does not depend on the number of active channels that the Goldman equation is preferred over other methods (4, 5, 8) which use the relative amplitudes of currents and the "independence principle" to determine permeability ratios.

Fig. 2 shows the same type of experiment with sodium and methylamine Ringer's. There are no measurable inward currents of methylamine ions. For all depolarizations above −50 mV there are clear transient outward currents. Evidently methylamine passes extremely poorly through sodium channels. It is not practical to measure a reversal potential because it is at a potential so negative that sodium channels do not open spontaneously. The reduction of the reversal potential on changing from sodium to methylamine is greater than 107 mV. After corrections for junction potentials and the attenuation artefact this figure becomes 123 mV. From Eq. (1) the permeability to methylamine is less than 0.006 of that to sodium.

DISCUSSION

The new feature of this work is the quantitative evaluation of the permeabilities of hydrazine, hydroxylamine, and methylamine cations relative to sodium ions. The observation that hydrazine and hydroxylamine are permeant sodium substi-

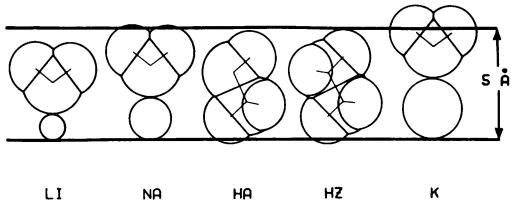

FIG. 3. Scale drawings showing interatomic distances and van der Waals surfaces for $Li \cdot H_2O$, $Na \cdot H_2O$, hydroxylamine, hydrazine, and $K \cdot H_2O$. All dimensions after Pauling (15). The horizontal heavy lines 5-Å apart represent the postulated space between oxygen atoms available for cations permeating the sodium channel. Methylamine would be virtually like hydrazine in this kind of drawing.

tutes is old. Lorente de Nó and coworkers (2) showed that these cations restore conducted action potentials to a sciatic nerve blocked by low sodium. Tasaki and coworkers (6, 7) found the same for membrane action potentials in squid giant axons. They also demonstrated that external hydrazine could give transient inward currents in the usual type of voltage clamp experiment. The inward currents were blocked by tetrodotoxin, a poison specific for the sodium channel. They also stated that methylamine is not as good a sodium substitute as hydroxylamine.

My experiments show that hydroxylamine cations pass through sodium channels about as easily as sodium ions and that hydrazine cations pass only slightly less easily. On the other hand, methylamine cations are virtually impermeant. It is instructive to consider why methylamine differs so much from the other two organic cations. The three molecules have a cationic ammonium group $-NH_3^+$ attached to an –OH, an $-NH_2$, or a $-CH_3$ group. The bond lengths N–O, N–N, and N–C are 1.45 ± 0.02 Å and the bond lengths O–H, N–H, and C–H are 1.04 ± 0.04 Å. Their largest outside (van der Waals) dimension ranges from 5.2 to 5.3 Å. Hence, the three cations are almost geometrically congruent except for a difference of one or two hydrogen atoms. If size and shape were the only criterion, these organic cations should have identical permeabilities.

Methylamine differs from the permeant cations in the inability of its methyl group to form hydrogen bonds. Hydrogen bonds are relatively weak bonds which can form between an oxygen atom (the acceptor) in any chemical compound and –OH or –NH functions (the donor) on the same or a different compound (15). In forming the bond, the acceptor oxygen may penetrate up to 0.9 Å closer to the donor hydrogen than in a van der Waals contact.

To explain the impermeability of methylamine, I postulate that the sodium channel provides at least one oxygen as a hydrogen bond acceptor about 5 Å from a negative charge which attracts the cationic part of the permeating particle. The negative charge must also be an oxygen of an oxygen acid, as no other atom can bear a stable negative charge in the functional pH range from 5 to 10 (14). In this model, compounds unable to donate a hydrogen bond to the acceptor oxygen while simultaneously lying against the negative site fail to permeate: Methylamine fails and hydrazine and hydroxylamine succeed.

How can the sodium ion be fitted into this model? The crystal radius of a sodium ion is only 0.95 Å and the ion has no hydrogens for hydrogen bonding (15). The sodium ion can be made to look like hydrazine and hydroxylamine by adding to it a water molecule. Fig. 3 is a scale drawing of these ions with their van der Waals radii. The $H_2O \cdot Na$ complex provides hydrogen-bondable hydrogens 5 Å from the contact margin of the cation. The orientation of water with respect to the cation in Fig. 3 is one which is postulated in many of the physicochemical descriptions of hydrated ions in solution because it minimizes the electrical energy of the water dipole (16). I suggest that this bimolecular arrangement is a component of the critical complex at the moment when a sodium ion passes the selectivity filter of the sodium channel. Very probably more than one water molecule is in the critical complex, but these experiments do not resolve that question.

In conclusion, the relative permeability of cations is explained by supposing that the sodium channel contains (*1*) an ionized oxygen acid which attracts the cation to it, and (*2*) some other oxygen group or groups, about 5 Å away, which must receive hydrogen bonds before the cation can pass. As will be shown in a later paper, this hypothesis covers all known permeant and impermeant cations. Alkali-metal cations must be accompanied by at least one water molecule to fit. Fig. 3 shows this complex for lithium, sodium, and potassium ions. Because the first two ions are known to permeate equally well and potassium ions very poorly, I further suppose that the sodium channel favors small-ion–water complexes and discriminates against larger ones. Thus, the sodium channel probably passes partially hydrated alkali-metal ions in a polar environment and uses size as a criterion to distinguish them. This description is intended to apply strictly to sodium channels of axons. Whether it can be used for other examples of ionic selectivity can only be determined by further studies.

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