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Water splitting in the cell membrane. Possible effects on membrane conductance and intracellular pressure

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It is shown that hydrogen and hydroxyl ions, produced in protonation and deprotonation reactions between ionisable groups in a cell membrane and water, will leave the reaction sites in opposite direction, rather than recombine with them when the electric field strength is equal to the mean value present in squid nerve at the resting potential. It is calculated that this effect could influence the conductance and swelling of intramembrane and sub-axolemmal protein during an inward current pulse, if the hydrogen ions combine with the acidic groups of the macromolecules.

Recent work by Tasaki and coworkers [1–4] has shown that water crosses the cell membrane, during an action potential, causing a transient increase in the axon diameter and the intracellular pressure. The peak in the intracellular pressure coincides with that of the action potential, suggesting that the mechanical and electrical variations have a common physicochemical origin. The results suggest that the intracellular pressure and the membrane conductance both depend on the water content of intramembrane and subaxolemmal protein.

The authors explained the data by a theory in which the water content of the protein was assumed to increase, when calcium counterions are replaced by potassium or sodium. It was suggested that the protein could be converted from a compact dehydrated state in which calcium ions are the counterions, at the resting potential, to a swollen hydrated state, in which much of the calcium is replaced by potassium and sodium, at the peak of the action potential. The swelling would be accompanied by an increase in membrane conductance, since the permeability of a polyelectrolyte to ions, increases with its water content. The authors [3] also noted that hydrogen ions could influence the swelling of the protein, since polyelectrolytes shrink when K^+ or Na^+ counterions are replaced by protons. They predicted that hydrogen ions would flow into the protein during an inward current pulse. However, it was uncertain whether the increase in proton concentration would be sufficient to cause a pronounced shrinkage of the molecules. We reconsider the proton question in this report.

In their paper, Tasaki and Iwasa [3] assumed that the protons which accumulate in the macromolecules, originate in the extracellular solution. Actually another source of hydrogen (or hydroxyl) ions is likely to be more important, namely the deprotonation (or protonation) reactions between ionisable groups of molecules in the membrane and water. The reactions have the form

$$B + H_2O \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} BH^+ + OH^- \quad (1a)$$

$$BH^+ + H_2O \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} B + H_3O^+ \quad (2a)$$

for basic groups, B, and

$$A^- + H_2O \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} AH + OH^- \tag{1b}$$

$$AH + H_2O \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} A^- + H_3O^+ \tag{2b}$$

for acidic groups, AH. The hydrogen and hydroxyl ions which are produced in these reactions, will leave the ionisable groups in opposite directions, rather than recombine with them, when the gradient in electrostatic potential is sufficiently large. Consequently, during inward current flow, there can be a migration of protons from the sites towards the axolemmal region, and of hydroxyl ions, towards the outside solution.

An effect of this type, known as water splitting, occurs when current flows through ion-exchange membranes [5,6]. When it takes place one of the boundary solutions becomes acidic and the other basic. It is noteworthy that the aqueous ions which contribute to the pH change originate almost exclusively in proton transfer reactions between ionisable groups and water in the membrane surface [7–10]. Only a small number originate in ordinary water dissociation in the boundary solutions. This suggests that water splitting might be more important in proton transport across biological membranes than the movement of hydrogen ions between the boundary solutions.

It is also noteworthy that water splitting can influence the degree of swelling, and hence the electrical resistance, of ion-exchange membranes, if the functional groups are weak acids or bases. For example, Negev Institute membranes, with tertiary alkyl amino groups, undergo a 10^5-fold increase in resistance and a 24% decrease in area, when fixed charge in the interior is neutralised by hydroxyl ions, as in water splitting [10]. The parameters alter because the charged groups are converted to the neutral form. This leads to the film becoming less hydrophilic so that it loses water to the boundary solutions.

It seems possible that water splitting could have a similar influence on the swelling and electrical resistance of intramembrane and sub-axolemmal protein in biological systems, if, as suggested by Tasaki and Iwasa [3], inward moving protons neutralise negatively charged groups of the macromolecules. We examine this possibility in the following.

We first show that water splitting can originate at ionisable groups in a cellular membrane when the electric field at the sites is equal to the mean value present in squid nerve at the resting potential.

Consider a region where the concentration of membrane bound acidic groups is C. Let C^- denote the concentration of sites which are in the charged form. Hydrogen (or hydroxyl) ions, produced in Reaction 2b (or 1b), between the sites and water, recombine with the groups in an average time $\tau_{chem} = 1/k_{-2}C^-$ (or $1/k_{-1}(C - C^-)$). The average displacement of the hydrogen ions at the end of this period would be zero, if the electric field $(-dV/dx)$ were zero. If the field were finite the ions would be displaced an average distance d_H, in the direction of $(-dV/dx)$ given by

$$d_H = u_H \frac{dV}{dx} \tau_{chem} = u_H \frac{dV}{dx} / k_{-2} C^- \tag{3}$$

where u denotes mobility. The factor u_H/k_{-2} can be estimated if the recombination reaction is diffusion controlled. The rate constant k_{-2} is then proportional to u_H [11,12] and u_H/k_{-2} is similar in magnitude to the free solution value. In water the rate constants of diffusion controlled reactions involving protons or hydroxyl ions are commonly in the range $(1–5) \cdot 10^7$ $m^3 \cdot mol^{-1} \cdot s^{-1}$, while $u_H = 3.6 \cdot 10^{-7}$ $m^2 \cdot V^{-1} \cdot s^{-1}$. A rough estimate for u_H/k_{-2} is therefore, $1.4 \cdot 10^{-14}$ $mol \cdot m^{-1} \cdot V^{-1}$.

Suppose now that the mean spacing of the sites is 2 nm, so that $C^- \lesssim C = 0.2$ M, and that $dV/dx = 10^7$ $V \cdot m^{-1}$, which is the average value in squid nerve at the resting potential. The protons would then travel an average distance d_H of approx. 0.7 nm, towards the cell interior, during the recombination time τ_{chem}, using Equation 3. Since this distance is comparable to the spacing of the fixed charges, we have, that the likelihood that a proton moves inwards before it recombines with an acidic group, is substantially greater than it is when the electric field is zero.

The same type of analysis can be applied to the hydroxyl ions which are produced in Reaction 1b, and to the hydroxyl and hydrogen ions which

would be produced in Reactions 1a and 2a, in a region with basic groups. One finds for both acid and basic regions, and for mixed acid/base regions, that when $dV/dx = 10^7\ V \cdot m^{-1}$, there is a substantial increase in the number of hydrogen ions which migrate inwards, and hydroxyl ions which migrate outwards, from the sites. When the ions are in electrochemical equilibrium these flows are cancelled by oppositely directed flows of aqueous ions towards the sites, under their concentration gradients. On the other hand, if the net electrochemical forces are finite and in the same direction as the electric forces, as in the cell membrane, the movement of hydrogen and hydroxyl ions away from the sites will exceed the backflows and water splitting will be observed.

We wish to determine whether water splitting could occur sufficiently quickly for the protons to neutralise the acidic groups of protein molecules during an inward current pulse. It is first necessary to estimate an upper limit for the rate of production of the aqueous ions in the proton transfer reactions.

The expressions for the pseudo-unimolecular forward rate constants k_1' and k_2' of Reactions 1b and 2b (or 1a or 2a) are

$$k_1' = k_{-1} \cdot 10^{-(14 - pK_a)} \tag{4a}$$

$$k_2' = k_{-2} \cdot 10^{-pK_a} \tag{4b}$$

It is seen that the rate at which the aqueous ions are produced is a maximum when the pK_a is 7 and that k_1' and k_2' both exceed $10^3\ s^{-1}$, for a group with a pK_a of 7, in free solution. The values of k_1' and k_2' for an ionisable group in a membrane would depend on the profiles for the dielectric constant and the aqueous ion mobilities. In the case of ion exchange membranes it appears that the aqueous ions are produced at a faster rate at groups in the surfaces than they are produced at groups with the same pK_a but in free solution [7,8]. This suggests that there might be groups in the cell membrane with a pK_a of 7, for which k_1' and k_2' exceed the free solution value of around $10^3\ s^{-1}$. Since the diffusion times of water and ions through the membrane, and the time required for hydrogen ions to combine with the acidic groups, are all likely to be much smaller than 10^{-4} s, water splitting could occur sufficiently quickly at such groups, for the protons to neutralise the acidic groups of protein molecules, if it continued for about 1 ms or longer. It follows, if the model of Tasaki and co-workers is correct, that water splitting could be a factor which influences the membrane conductance and the intracellular pressure during long inward current pulses. It might also influence the parameters during the repolarising phase of the action potential.

A test for the effect would be to confirm whether hydroxyl ions leave the membrane, in the region of the sodium or potassium channels at large negative voltages. It might be possible to detect the ions using a pH-sensitive fluorescent material.

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