Surface Potentials and the Calculated Selectivity of Ion Channels

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ABSTRACT Ion channels catalyze the transport of ions across biological membranes. A proper understanding of ion-channel functioning is essential to our knowledge of cell physiology, and, in this context, ion-channel selectivity is a key concept. The extent to which a channel permeates two ion species, a and b, is expressed by the permeability ratio, P_a/P_b. This paper addresses a complication in the calculation of P_a/P_b that is related to the existence of surface potentials (ψ) and that so far has not been fully appreciated. This paper shows the rather surprising effect of ψ on the calculated P_a/P_b of a channel that is permeable to two ion species of different valence. If we ignore ψ, we conclude, for instance, $P_a > P_b$. If we implement ψ in the calculation of P_a/P_b, we may, however, conclude exactly the reverse, i.e., $P_a < P_b$. Because electrostatic potentials arise at the surface of essentially all biological membranes, this paper argues for a more critical evaluation of ion channel selectivity measurements.

INTRODUCTION

Ion channels are enzymes (Eisenberg, 1990; Jan and Jan, 1992). Despite the difference between ion channels or electroenzymes (Laüger, 1991) and ordinary enzymes, which catalyze the conversion of one chemical form into another, the two classes of enzymes have an important characteristic in common. Like their counterparts, ion channels show substrate specificity or, in "channology" (Eisenberg, 1990), ion selectivity. The ion selectivity is one, if not the most important, characteristic of an ion channel (Cornish-Bowden, 1984). The permeability ratio P_a/P_b is a measure of the selectivity of an ion channel and reflects the ability to which the channel can discriminate between two ion species a and b. The aim of this study is to show the effect of surface potentials on the calculated P_a/P_b in the case where the two ion species, a and b, are of different valence.

Surface potentials (ψ) arise from surface charges at the membrane surface, notably the net negative charge of acidic phospholipids (McLaughlin et al., 1971; Latorre et al., 1992). In addition, acidic or basic amino acids of proteins embedded in the phospholipid bilayer may contribute to the overall surface charge (Green and Anderson, 1991; Latorre et al., 1992; Naranjo et al., 1994; Elinder and Århem, 1999). The question therefore is not whether biological membranes do have a fixed net negative charge, but to what extent. Estimates of the electrostatic charge of biological membranes range from 0.0025 to 0.01 electronic charges per $Å^2$. Depending on the ionic strength of the solution and the extent to which charges are neutralized by ion binding (Hille et al., 1975; MacLaughlin, 1989), a negative surface charge gives rise to a surface potential, typically in the range of -30 to -90 mV (Hille, 1994). Surface potentials affect the distribution of ion species near the membrane surface. A net negative surface charge, for instance, attracts divalent cations more than monovalent cations but repels anions. Because of this, surface potentials play an important role in mineral rhizotoxicity (Yermiyahu et al., 1997). The redistribution of ions at the charged surface may also alter the ion permeation through the channel (Dani, 1986; Jordan, 1987; Cai and Jordan, 1990) and this, in turn, may change the single-channel conductance (Rostovtseva et al., 1998; Banach et al., 2000). In addition, because ψ offsets the potential actually experienced by the voltage sensor, the gating kinetics of the channel may be affected (Hille et al., 1975; Cens et al., 1998; Elinder and Århem, 1999). Although effects of ψ on ion-channel behavior have been widely acknowledged (Begenisich, 1975), effects of ψ on P_a/P_b are generally ignored. The reason that the calculation of P_a/P_b is very rarely corrected for the effect of ψ is that electrodes do not sense surface potentials because they are always positioned in the bulk phases of the solutions (Hille, 1994). Consequently, E_{rev} is not affected by ψ, and, during an electrophysiological recording, the existence of ψ can neither be verified nor falsified, at least not directly. Indirect evidence for the presence of ψ can be obtained from the relative insensitivity of the single-channel conductance to low concentrations of the permeable ion species (Banach et al., 2000). To my knowledge, Lewis (1979) has been one of the very few who calculated the effect of ψ on P_a/P_b, although she used a slightly different approach. She fitted her data assuming a permeability for three ion species and found that the calculated P_{Ca}/P_{Na} was sensitive to ψ_{ext}. The analysis given here shows that the effect of ψ on P_a/P_b is far more reaching and that the apparent selectivity of a channel for the two ion species, a and b, can even reverse.

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RESULTS

The most common approach to determine the selectivity of an ion channel relies on the measurement of the potential of net zero current, the so-called reversal potential, E_{rev} (Eisenman and Horn, 1983). The value of P_a/P_b is related to the

location of E_{rev} along the voltage axis in respect to the theoretical equilibrium or Nernst potentials of each individual ion species. P_a/P_b can be derived from the Goldman–Hodgkin–Katz (GHK) current equation (Hille, 1994), and is given by

$$\frac{P_a}{P_b} = -\frac{z_b^2(b_{cyt} - b_{ext}\exp(-z_bE_{rev}F/RT))}{z_a^2(a_{cyt} - a_{ext}\exp(-z_aE_{rev}F/RT))} \times \frac{(1 - \exp(-z_aE_{rev}F/RT))}{(1 - \exp(-z_bE_{rev}F/RT))}, \quad (1)$$

where a_{cyt}, a_{ext}, b_{cyt}, and b_{ext} refer to the bulk activities of ion species a with valence z_a and ion species b with valence z_b (RT/F = 25.3 mV at 20°C). E_{rev} is usually measured under highly asymmetrical ionic conditions, i.e., with a high salt concentration at one side of the membrane and a low salt concentration at the other. The reason is that, the more distance between the Nernst potentials (E_N) of the permeable ion species, the more reliable the determination of the ion selectivity. To illustrate this point, suppose we study an ion channel in the whole-cell configuration with 100 mM KCl in the pipette solution and 50 mM KCl in the bath solution (activities), resulting in an E_K and E_{Cl} of −17.5 and 17.5 mV, respectively. Imagine that, under these conditions, we measure an E_{rev} of −11 mV. Then, according to Eq. 1, we calculate a K^+ over Cl^- selectivity (P_K/P_{Cl}) of 4.8. Furthermore, suppose an error in the determination of E_{rev} of ±3 mV, implying that P_K/P_{Cl} lies between 2.8 and 9.5. Next, we lower the KCl in the bath solution from 50 to 10 mM, resulting in an E_K of −58.2 mV and an E_{Cl} of 58.2 mV. Imagine that we now measure an E_{rev} of −30 mV. We calculate a very similar P_K/P_{Cl} of 4.7. Assuming a similar error of ±3 mV in the determination of E_{rev}, we conclude however that, in this case, P_K/P_{Cl} falls between 4.0 and 5.7, a range almost four times as narrow as with the 50 mM KCl bath solution. In other words, the steeper the ion gradients of the two permeable ion species, and thus the more distance between E_a and E_b (in the two examples given 35 versus 116.4 mV), the less sensitive P_a/P_b for errors in E_{rev}. This procedure for the determination of E_{rev} with a solution of low ionic strength at one side of the membrane may however be prone to a serious error that is related to the existence of surface potentials.

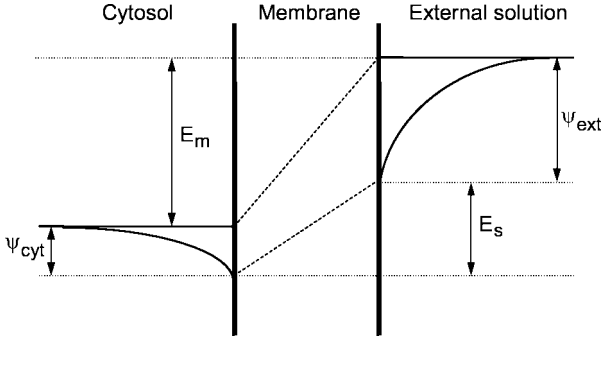

FIGURE 1 Schematic representation of the way surface potentials (ψ) offset the potential across the channel pore. The membrane potential (E_m) is defined as the difference between the bulk phase potential at the cytosolic side and the bulk phase potential at the external side of the membrane ($E_m = E_{cyt} - E_{ext}$). The potential actually experienced by the voltage sensor of the channel (E_s) and E_m are related by $E_s = E_m + \psi_{cyt} - \psi_{ext}$ (see Eq. 2). Adapted after Hille et al. (1975) and Hille (1994).

Surface potentials add to the existing potential between the bulk solutions at either side of the membrane, i.e., the membrane potential (Hille et al., 1975; Green and Anderson, 1991). In the presence of a surface potential at the cytosolic side (ψ_{cyt}) and at the external side of the membrane (ψ_{ext}), the actual voltage drop along the channel pore (E_s) is given by (Fig. 1),

$$E_s = (E_{cyt} + \psi_{cyt}) - (E_{ext} + \psi_{ext}) = E_m + \psi_{cyt} - \psi_{ext}, \quad (2)$$

where E_{cyt} and E_{ext} refer to the potential of the bulk phases of the solutions, and the membrane potential (E_m) is defined as $E_{cyt} - E_{ext}$. In the presence of ψ, ions distribute at the charged surface according to a Boltzmann expression (McLaughlin et al., 1971; Hille et al., 1975),

$$c_s = c\,\exp(-z\psi F/RT), \quad (3)$$

where c_s refers to the ion concentration at the membrane surface and c to the concentration in the bulk phase of the solution. To calculate P_a/P_b in the presence of surface potentials, we use the ionic conditions at the membrane surface rather than those prevailing in the bulk solutions. We therefore substitute Eqs. 2 and 3 into Eq. 1 and, after some rearrangements, we obtain the expression,

$$\frac{P_a}{P_b} = -\frac{z_b^2(b_{cyt} - b_{ext}\exp(-z_bE_{rev}F/RT))}{z_a^2(a_{cyt} - a_{ext}\exp(-z_aE_{rev}F/RT))} \times \frac{(1 - \exp(-z_a(E_{rev} + \psi_{cyt} - \psi_{ext})F/RT))}{(1 - \exp(-z_b(E_{rev} + \psi_{cyt} - \psi_{ext})F/RT))} \times \exp((z_a - z_b)\psi_{cyt}F/RT). \quad (4)$$

Note that, if $z_a = z_b$, the last two terms on the right-hand side of Eq. 4 are canceled, resulting in an expression for P_a/P_b independent of ψ, implying that, only if $z_a \neq z_b$, does ψ have an effect on P_a/P_b. The differential effect of ψ on the distribution of ion species of different valence is indeed the reason that ψ does have an effect on the calculated value of P_a/P_b. Also note that, if $\psi_{cyt} = \psi_{ext}$, ψ still has an effect on P_a/P_b. In that particular case, P_a/P_b equals $(P_a/P_b)_{\psi=0}\exp((z_a - z_b)\psi_{cyt}F/RT)$, where $(P_a/P_b)_{\psi=0}$ represents P_a/P_b with $\psi = 0$ mV (Eq. 1).

We now return to the example of a channel that permeates K^+ and Cl^-. With 100 and 10 mM KCl in the pipette and bath, respectively, E_{rev} was assumed to be −30 mV. Ignoring surface potentials and according to Eq. 1 (or Eq. 4

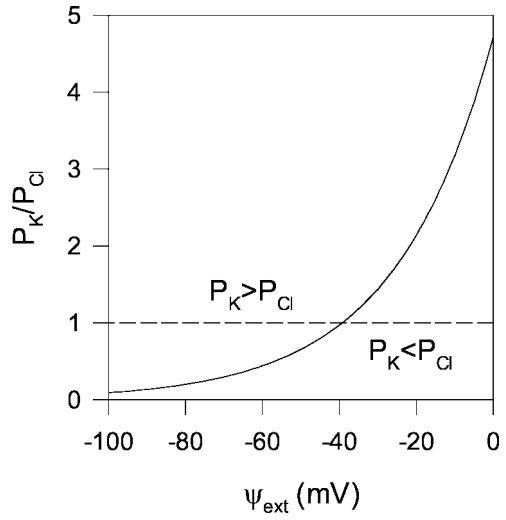

FIGURE 2 Simulation of the calculated permeability ratio P_K/P_{Cl} for an ion channel that permeates K^+ and Cl^-. Cytosolic and external KCl activities were assumed to be 100 and 10 mM, respectively. Values of P_K/P_{Cl} were calculated as a function of ψ_{ext}, according to Eq. 4 with ψ_{cyt} = 0 mV and assuming an E_{rev} of −30 mV. Note that the calculated selectivity of the channel depends on the magnitude of ψ_{ext}. If $\psi_{ext} > -40$ mV, we conclude $P_K > P_{Cl}$; on the contrary, if $\psi_{ext} < -40$ mV, we conclude exactly the opposite, i.e., $P_K < P_{Cl}$.

with $\psi_{cyt} = \psi_{ext} = 0$ mV), we calculated a P_K/P_{Cl} of 4.7 and therefore concluded that the channel is more permeable to K^+ than to Cl^-. Now consider a negative surface potential at the external side of the membrane, i.e., at the side with the solution of low ionic strength. As before, we still measure an E_{rev} of −30 mV but we repeat the calculation of P_K/P_{Cl} for different values of ψ_{ext}, according to Eq. 4 with ψ_{cyt} = 0 and $\psi_{ext} \neq 0$. The result is shown in Fig. 2. This procedure not only results in significantly smaller values of P_K/P_{Cl} but, more importantly, at ψ_{ext} more negative than −40 mV, the value of $P_K/P_{Cl} < 1$ and falls to a value as low as 0.09 at $\psi_{ext} = -100$ mV. This value is no less than fifty times lower than the value of 4.7. This is an important observation. It means that, depending on the magnitude of ψ_{ext}, the correction for the effect of ψ_{ext} on P_K/P_{Cl} determines whether we conclude that the channel is more selective for K^+ than for Cl^- or vice versa.

DISCUSSION

Although ψ can have a large effect on the calculated P_a/P_b, it should be realized that surface potentials do not change the intrinsic selectivity of an ion channel. In this respect, the effect of ψ described here is essentially different from experiments in which they actually measured changes of E_{rev} after changing the pH or ionic strength of the solution (Borisenko et al., 2000; Trexler et al., 2000). However, as is obvious from Fig. 2, if we ignore ψ, we may draw an erroneous conclusion as far as the selectivity of the channel is concerned. It should also be emphasized that, in the presence of ψ, the driving force for ion movement, i.e., the difference in electrochemical potential ($\Delta\mu$), remains unaffected. By substituting Eq. 3 into the Nernst equation, it can be verified easily that E_N for each ion species shifts by the same factor as E_m (Eq. 2), i.e., $\psi_{cyt} - \psi_{ext}$. Because the changes of E_m and E_N have an opposite effect on $\Delta\mu$, the change of E_m is exactly balanced by the change of E_N and, as a result, $\Delta\mu$ remains the same. Regardless of the presence and magnitude of ψ, the current direction of each individual ion species thus remains solely determined by $\Delta\mu$ between the bulk solutions. In the example of Fig. 2, for instance, at an E_m of −100 mV, the flux of K^+ is always inwardly directed and the flux of Cl^- is always outwardly directed, irrespective of ψ_{cyt} and ψ_{ext}. Assuming that effects of ψ_{ext} can safely be ignored and, according to the calculated P_K/P_{Cl} of 4.7, we conclude that the overall current at −100 mV is dominated by an influx of K^+. In contrast, if there is evidence that $\psi_{ext} < -40$ mV, we conclude that the correct P_K/P_{Cl} is not 4.7 but instead <1. Consequently, we conclude that an efflux of Cl^- rather than an influx of K^+ dominates the current at −100 mV. Depending on ψ_{ext}, the contribution of each ion species to the overall current might thus be quite different from that concluded from the apparent P_K/P_{Cl} with ψ_{ext} = 0 mV. This example demonstrates the profound effect of ψ on P_a/P_b and its impact on the interpretation of electrophysiological data, at both the biophysical and physiological level. Similar effects as described here arise with the calculation of the permeability ratio of a channel that is permeable to monovalent and divalent cations, for instance, Na^+ and Ca^{2+}. As is obvious from Eq. 4, the extent to which P_a/P_b is affected by ψ depends not only on the absolute values of z_a and z_b but also on the difference between the two, $z_a - z_b$ (in the case $\psi_{cyt} \neq 0$).

What does this all mean in practice? Although reversal potential measurements are a standard routine in electrophysiology, does this imply that such selectivity measurements are inherently unreliable? The answer to this question is yes and no. No, because the effect of ψ on P_a/P_b only arises if the two ion species are of different valence. Yes, because it is safe to assume that practically all biological membranes bear, to more or less extent, a net electrical charge. Given this and dependent on the ionic strength of the solution, the existence of surface potentials is the rule rather than the exception. Even at relatively low values of ψ, P_a/P_b will be affected. It this context, note in Fig. 2 that the less negative ψ_{ext}, the steeper the dependence of P_a/P_b on ψ_{ext}, i.e., the more sensitive P_a/P_b to ψ_{ext}. The important point to realize is that the effect of ψ on P_a/P_b goes unnoticed. Whenever using solutions of low ionic strength, the first step to minimize the chance of a misinterpretation of the data is to be aware of the existence of ψ and realize its effect on P_a/P_b. To reduce ψ, it may seem obvious to simply increase the cation content of the solutions, notably by

adding divalent cations, which effectively screen negative surface charges. However, this option has two important drawbacks. First, during selectivity measurements, one wants to minimize the number of permeable ion species. Second, increasing the Ca^{2+} or Ba^{2+} concentrations may induce unwanted side effects, for instance, a block of the channel of interest (Green and Anderson, 1991). More promising, therefore, seems to either measure (McLaughlin et al., 1970; Brauer et al., 2000) or make an estimate of the surface charge and calculate ψ (Peitzsch et al., 1995) or, alternatively, measure ψ directly (Kraayenhof et al., 1993) and account for its effect according to Eq. 4.

Despite its shortcomings as far as the constant field assumption (Syganow and Kitzing, 1999, but see Roux, 1999) and ion–ion interactions are concerned (Eisenman and Horn, 1983; Hille, 1994), the GHK equation generally still is the starting point for the calculation of P_a/P_b. The effect of ψ on P_a/P_b described here adds another complication to the interpretation of calculated values of P_a/P_b. As detailed knowledge of the three- dimensional structure of channel proteins progresses (Doyle et al., 1998; Roux and MacKinnon, 1999; Miller, 2000), molecular dynamics studies based on the energy profile of the channel pore will become more and more feasible. It will be of great interest to see whether, eventually, such simulation studies may appear more successful for the determination of the true or correct ion selectivity of ion channel proteins (Åqvist and Luzhkov, 2000).

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