

## 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 ( $\psi$ ) and that so far has not been fully appreciated. This paper shows the rather surprising effect of  $\psi$  on the calculated  $P_a/P_b$  of a channel that is permeable to two ion species of different valence. If we ignore  $\psi$ , we conclude, for instance,  $P_a > P_b$ . If we implement  $\psi$  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 (Läuger, 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 ( $\psi$ ) 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  $\text{\AA}^2$ . 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  $\psi$  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  $\psi$  on ion-channel behavior have been widely acknowledged (Begenisich, 1975), effects of  $\psi$  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  $\psi$  is that electrodes do not sense surface potentials because they are always positioned in the bulk phases of the solutions (Hille, 1994). Consequently,  $E_{\text{rev}}$  is not affected by  $\psi$ , and, during an electrophysiological recording, the existence of  $\psi$  can neither be verified nor falsified, at least not directly. Indirect evidence for the presence of  $\psi$  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  $\psi$  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  $P_{\text{Ca}}/P_{\text{Na}}$  was sensitive to  $\psi_{\text{ext}}$ . The analysis given here shows that the effect of  $\psi$  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.

### 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,  $E_{\text{rev}}$  (Eisenman and Horn, 1983). The value of  $P_a/P_b$  is related to the

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location of  $E_{\text{rev}}$  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

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

where  $a_{\text{cyt}}$ ,  $a_{\text{ext}}$ ,  $b_{\text{cyt}}$ , and  $b_{\text{ext}}$  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  $20^\circ\text{C}$ ).  $E_{\text{rev}}$  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  $E_{\text{Cl}}$  of  $-17.5$  and  $17.5$  mV, respectively. Imagine that, under these conditions, we measure an  $E_{\text{rev}}$  of  $-11$  mV. Then, according to Eq. 1, we calculate a  $\text{K}^+$  over  $\text{Cl}^-$  selectivity ( $P_K/P_{\text{Cl}}$ ) of 4.8. Furthermore, suppose an error in the determination of  $E_{\text{rev}}$  of  $\pm 3$  mV, implying that  $P_K/P_{\text{Cl}}$  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  $E_{\text{Cl}}$  of  $58.2$  mV. Imagine that we now measure an  $E_{\text{rev}}$  of  $-30$  mV. We calculate a very similar  $P_K/P_{\text{Cl}}$  of 4.7. Assuming a similar error of  $\pm 3$  mV in the determination of  $E_{\text{rev}}$ , we conclude however that, in this case,  $P_K/P_{\text{Cl}}$  falls between 4.0 and 5.7, a range almost four times as narrow as with the 50 mM KCl

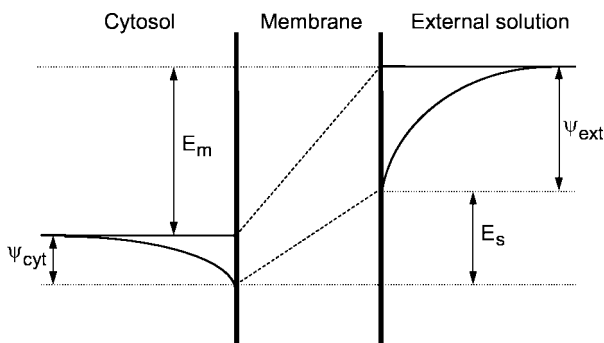


FIGURE 1 Schematic representation of the way surface potentials ( $\psi$ ) offset the potential across the channel pore. The membrane potential ( $E_m$ ) is defined as the difference between the bulk phase potential at the cytosolic side and the bulk phase potential at the external side of the membrane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

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  $E_{\text{rev}}$ . This procedure for the determination of  $E_{\text{rev}}$  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.

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 ( $\psi_{\text{cyt}}$ ) and at the external side of the membrane ( $\psi_{\text{ext}}$ ), the actual voltage drop along the channel pore ( $E_s$ ) is given by (Fig. 1),

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

where  $E_{\text{cyt}}$  and  $E_{\text{ext}}$  refer to the potential of the bulk phases of the solutions, and the membrane potential ( $E_m$ ) is defined as  $E_{\text{cyt}} - E_{\text{ext}}$ . In the presence of  $\psi$ , 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,

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

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  $\psi$ , implying that, only if  $z_a \neq z_b$ , does  $\psi$  have an effect on  $P_a/P_b$ . The differential effect of  $\psi$  on the distribution of ion species of different valence is indeed the reason that  $\psi$  does have an effect on the calculated value of  $P_a/P_b$ . Also note that, if  $\psi_{\text{cyt}} = \psi_{\text{ext}}$ ,  $\psi$  still has an effect on  $P_a/P_b$ . In that particular case,  $P_a/P_b$  equals  $(P_a/P_b)_{\psi=0} \exp((z_a - z_b)\psi_{\text{cyt}}F/RT)$ , 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  $\text{K}^+$  and  $\text{Cl}^-$ . With 100 and 10 mM KCl in the pipette and bath, respectively,  $E_{\text{rev}}$  was assumed to be  $-30$  mV. Ignoring surface potentials and according to Eq. 1 (or Eq. 4

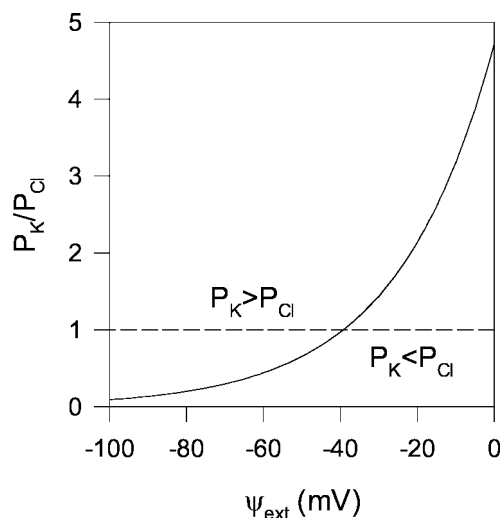


FIGURE 2 Simulation of the calculated permeability ratio  $P_K/P_{Cl}$  for an ion channel that permeates  $K^+$  and  $Cl^-$ . Cytosolic and external KCl activities were assumed to be 100 and 10 mM, respectively. Values of  $P_K/P_{Cl}$  were calculated as a function of  $\psi_{ext}$ , according to Eq. 4 with  $\psi_{cyt} = 0$  mV and assuming an  $E_{rev}$  of  $-30$  mV. Note that the calculated selectivity of the channel depends on the magnitude of  $\psi_{ext}$ . 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  $\psi_{ext}$ , according to Eq. 4 with  $\psi_{cyt} = 0$  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  $\psi_{ext}$  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  $\psi_{ext}$ , the correction for the effect of  $\psi_{ext}$  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  $\psi$  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  $\psi$  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  $\psi$ , 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  $\psi$ , 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  $\psi$ , 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  $\psi_{cyt}$  and  $\psi_{ext}$ . Assuming that effects of  $\psi_{ext}$  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  $\psi_{ext}$ , 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  $\psi_{ext} = 0$  mV. This example demonstrates the profound effect of  $\psi$  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  $\psi$  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  $\psi$  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  $\psi$ ,  $P_a/P_b$  will be affected. In this context, note in Fig. 2 that the less negative  $\psi_{ext}$ , the steeper the dependence of  $P_a/P_b$  on  $\psi_{ext}$ , i.e., the more sensitive  $P_a/P_b$  to  $\psi_{ext}$ . The important point to realize is that the effect of  $\psi$  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  $\psi$  and realize its effect on  $P_a/P_b$ . To reduce  $\psi$ , 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  $\text{Ca}^{2+}$  or  $\text{Ba}^{2+}$  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  $\psi$  (Peitzsch et al., 1995) or, alternatively, measure  $\psi$  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  $\psi$  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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