[6] Correction for Liquid Junction Potentials in Patch Clamp Experiments

By ERWIN NEHER

Introduction

It is standard practice in patch clamp experiments to indicate membrane potentials with respect to the zero-current potential, as measured when the patch pipette is positioned near the cell but not yet sealed. This procedure can be considered as valid in the case of a cell-attached or inside-out patch measurement provided that the pipette contains a solution identical to that of the bath. Whenever these two solutions are different, however, there will be a liquid junction potential at the tip of the pipette during the determination of the zero-current potential; this complicates matters.[1-3]

Liquid junction potentials are due to different mobilities of ions at interfaces between different solutions.[4] Intuitively they can be understood as a result of some inbalance of charge, which results when the more mobile ion diffuses more rapidly across the concentration gradient at the interface. Liquid junction potentials are typically 2–12 mV for the solutions in general use in electrophysiological experiments.

In conventional microelectrode recordings the problem of liquid junction potentials is minimized by using concentrated KCl as the electrode filling solution. KCl is chosen because K^+ and Cl^- have almost equal ionic mobilities, and, as a consequence, liquid junction potentials are in the range of 1 mV or smaller. In patch clamping, on the other hand, the choice of solutions is dictated by the physiological requirements of the experiment. Although there are good reasons for tolerating liquid junction problems, the necessity remains to compensate for their effects. Unfortunately, authors of patch clamp papers only rarely describe in detail how they handle these problems.

Electrochemical Account of Patch Clamp Measurements

Strictly speaking the patch clamp measurement consists of two parts: (1) a reference measurement under current clamp conditions which results

[1] E. M. Fenwick, A. Marty, and E. Neher, *J. Physiol. (London)* **331**, 577 (1982).
[2] P. H. Barry, this series, Vol. 171, p. 678.
[3] P. H. Barry and J. W. Lynch, *J. Membr. Biol.* **121**, 101 (1991).
[4] J. O'M. Bockris and A. K. N. Reddy, "Modern Electrochemistry," Vol. 1. Plenum, New York, 1970.

in the zero-current potential and (2) a test measurement under voltage-clamp conditions, in which membrane current is measured as a function of applied voltage (after seal formation). Figure 1 (left-hand side) depicts schematically the reference measurement. It can be seen that the potential, V, which can be measured under zero-current conditions consists of three terms: V_{El1}, the electrode potential of the silver chloride electrode in the pipette; $-V_{LJ}$, the liquid junction potential; and $-V_{El2}$, the electrode potential of the bath electrode. V_{LJ} appears with negative polarity here, because the convention of Barry[2] is followed, which defines V_{LJ} as the potential of the bath solution with respect to the pipette solution. V_{El2} also has been denoted with negative polarity, since the orientation of the reference electrode is opposite to that of the pipette electrode.

Figure 1 (right-hand side) depicts the situation after formation of an inside-out patch. Any concentration gradients which gave rise to the V_{LJ} on the left-hand side now occur across the patch and give rise to the membrane currents of interest. It is immediately apparent that a potential of absolute magnitude V_{LJ} is being applied to the patch if V is left constant during patch formation. Thus, it is incorrect to accept the zero-current potential as the origin of the voltage axis. Concerning the polarity, considering, as in the above case (and throughout this chapter), V_{LJ} as the potential of the bath with respect to the inside of the pipette, the potential applied would be V_{LJ}, since, by convention, the potential of the inside-out patch is the negative of the pipette potential. Thus, the membrane potential V_M should be calculated from the reading provided by the patch-clamp

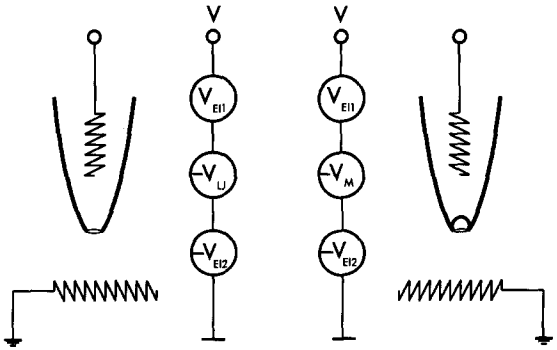

FIG. 1. Schematic representation of the reference measurement (left-hand side) and an inside-out patch measurement (test measurement, right-hand side). On the far left a patch pipette and the electrodes are depicted symbolically. Adjacent is a representation of the relevant electrochemical electromotive forces V_{El1}, $-V_{LJ}$, and $-V_{El2}$ (see text). For zero current the condition $V_{El1} - V_{LJ} - V_{El2} = V$ must be fulfilled. In the test measurement (right-hand side), $-V_{LJ}$ is replaced by the patch potential $-V_M$.

amplifier V according to

$$V_M = -V + V_{LJ} \tag{1}$$

for inside-out. The above treatment assumes that all the conditions stay constant in between the reference measurement and the test measurement and, in particular, that the electrode potentials are stable.

The arguments above also apply to the cell-attached configuration, except that the potential of the cell, V_c, has to be taken into account. With the usual conventions,[5] Eq. (1) then takes the form

$$V_M = V_c - V + V_{LJ} \tag{2}$$

for cell attached. In the outside-out configuration the absolute magnitude of the membrane potential is the same as in the inside-out case, but polarity is inverted:

$$V_M = V - V_{LJ} \tag{3}$$

for outside-out patch and small whole-cell.

The whole-cell configuration is equivalent to the outside-out case except for an additional problem. For the outside-out patch it has been assumed that there is no liquid junction potential between some residual cytoplasm attached to the membrane and the pipette solution, because concentration gradients at this junction are expected to equilibrate rapidly. In whole-cell measurements this may also be the case if small cells are employed. Small mobile ions equilibrate across the pipette orifice by diffusion within a few seconds in cells of 10–20 μm diameter.[6-8] However, for larger cells this may not be the case. The situation can be even more complex since the cytoplasm may contain immobile anions, giving rise to Donnan potentials. These complexities have been discussed in detail.[3-7]

Patch Clamp Amplifiers and Liquid Junction Potentials

Most patch clamp amplifiers provide separate settings for handling holding potential (V_{Hold}) and an offset. The sum of both is applied to the

[5] By convention, the liquid junction potential V_{LJ} is given as the potential of the bath with respect to that inside the pipette. Membrane potential V_M is given as that of the cytoplasmic side with respect to the extracellular side. The voltage reading V of the amplifier is set to zero in the zero-current condition by means of an offset. This offset stays fixed during the rest of the measurement.

[6] O. P. Hamill, A. Marty, E. Neher, B. Sakmann, and F. J. Sigworth, *Pfluegers Arch.* **391**, 85 (1981).

[7] A. Marty and E. Neher, *in* "Single-Channel Recording" (B. Sakmann and E. Neher, eds.), p. 107. Plenum, New York, 1983.

[8] M. Pusch and E. Neher, *Pfluegers Arch.* **411**, 204 (1988).

headstage, but only V_{Hold} is displayed on a meter and is available at an output. During the reference measurement V_{Hold} is set to zero, and the offset is adjusted for zero current. Then the test measurement is done at an appropriate V_{Hold} setting. Finally the correction as indicated in Eqs. (1)–(3) is applied. For convenience some amplifiers provide a switch-selectable operating mode (setup mode, search mode, or something similar) in which V_{Hold} is disenabled to avoid the necessity of repeatedly resetting V_{Hold} during cycles of reference and test measurements.

Alternatively, the following procedure can be adopted in order to avoid the *a posteriori* correction. The offset is adjusted for zero current during the reference while V_{Hold} is active and set to $-V_{LJ}$. Thereby the setting of V_{Hold} anticipates the potential that will be seen by the patch after seal formation (compare Fig. 1). This procedure exactly implies the corrections of Eqs. (1)–(3) if subsequently V_{Hold} readings are interpreted as $-V_M$ for inside-out and cell-attached configurations or as $+V_M$ for the other configurations.

Solution Changes: Puffer Pipettes, "Sewer Pipes," and U Tools

So far, it was assumed that the bath solution does not change in between reference and test measurements. Very often, however, it is desirable to change ionic concentrations in the bathing medium. In such cases it has to be strictly avoided that the reference electrode "sees" the solution change, particularly if it involves a change in chloride concentration, since V_{El2} (Fig. 1) depends strongly on chloride. Thus, the use of a salt bridge is indicated (but note the caveat on salt bridges below!). It may be simpler to change only the solution in the immediate vicinity of the cell by local perfusion using a puffer pipette, "sewer pipe" arrangement, or U tool. This also prevents concentration changes at the reference electrode. However, under these conditions, concentration gradients exist within the experimental chamber, giving rise to liquid junction potentials wherever they occur.[3]

The situation is depicted in Figure 2 for a whole-cell measurement. It turns out that

$$V_M = V - \Delta V_{El} - V_{2,1} \tag{4}$$

where $V_{2,1}$ is the liquid junction potential of solution 2 (close to the cell) with respect to the solution further away, the ΔV_{El} is $V_{El1} - V_{El2}$. If we call V_{ref} the potential applied to the pipette during the reference measurement (performed with the original solution) we see from Fig. 1 that

$$V_{ref} = -V_{LJ} + \Delta V_{El} \tag{5}$$

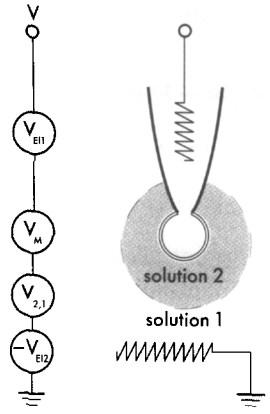

FIG. 2. Local perfusion. The shaded area (right) represents the milieu in the vicinity of a cell in which the bath solution (solution 1) has been replaced by a test solution (solution 2) through local perfusion. The left-hand side schematically gives the electrical potential differences involved.

From Eqs. (4) and (5) we obtain (after elimination of ΔV_{El})

$$V_M \simeq (V - V_{ref}) - V_{LJ} - V_{2,1}$$

During the reference measurement the amplifier offset had been adjusted, such that V_{ref} appears to be zero. Therefore

$$V_M = V - V_{LJ} - V_{2,1} \tag{6}$$

for whole-cell and outside-out patch recording, in analogy to Eq. (3). Thus the *a posteriori* correction is the sum of the two liquid junction potentials.

Let us assume for simplicity that the pipette solution contains predominantly potassium glutamate, that solution 2 is sodium glutamate, whereas solution 1 is Ringer's solution. In this instance, V_{LJ} and $V_{1,2}$ are similar in absolute magnitude, since in both cases the anion (glutamate) is much less mobile than the cation (K^+ or Na^+). However, with the conventions specified the polarities are different, thus the *a posteriori* correction for the part of the experiment performed in sodium glutamate is very small. The correction would be approximately -10 mV for measurements in Ringer's solution and -3 mV during local sodium glutamate perfusion. Disregarding these differences would lead to serious errors in the determination of permeability ratios. If, for instance, the shift of the reversal potential for a 10-fold change in $[Cl^-]$ is measured as 47 mV instead of 55 mV, one would estimate a P_{Cl}/P_{Glu} of 16 instead of 70 (based on the Goldman–Hodgkin–Katz equation).

For the inside-out configuration Eq. (1) would change by analogy to Eq. (6) to

$$V_M = -V + V_{LJ} + V_{2,1} \tag{7}$$

for inside-out patch. Similarly Eq. (2) would change to

$$V_M = V_c - V + V_{LJ} + V_{2,1} \tag{8}$$

for cell-attached, where V_c, the intracellular potential, has to be known for the condition of the local ionic milieu.

Using Salt Bridges

Careful investigators might prefer to avoid the problems associated with local solution changes for the reasons described above and use salt bridges. However, even with salt bridges a situation very similar to that described above may arise. The optimal salt bridge involves a compromise: on the one hand, it should contain 3 *M* KCl and should be "freely flowing" for a low liquid junction potential[9]; on the other hand, it should not contaminate the bath with KCl. The compromise very often is a relatively thin agar-filled piece of tubing, which at the beginning of the experiment contains KCl throughout. As the experiment proceeds, KCl diffuses out of the agar, and the interface between Ringer-like agar and KCl–agar recedes toward the interior of the bridge. If then a rapid solution change in the bath is performed, one is left essentially with a double-bridge arrangement as depicted in Fig. 3. If the electrode potentials and $V_{1,3}$ are corrected for during the reference measurement, exactly the same situation as discussed in the last section arises.

This means that a bridge, unless used with great care, is not much better than local perfusion. This point was already stressed in 1970 by Barry and Diamond[9] in the context of measurements of epithelial potentials and more recently by Barry and Lynch.[3] These authors also pointed out that electrochemically well-defined junctions in a physiological context can be obtained in two special cases. One is the biionic case, in which salts at equal concentrations are present at both sides of the junction, and either the cation or the anion is the same on both sides; the other one is the dilution case, in which the salts on both sides are identical but may have different concentrations. It can be shown that for these two cases the total junction potential does not depend on the particular concentration profile and thus does not change appreciably with time.[9]

[9] P. H. Barry and J. M. Diamond, *J. Membr. Biol.* **3**, 93 (1970).

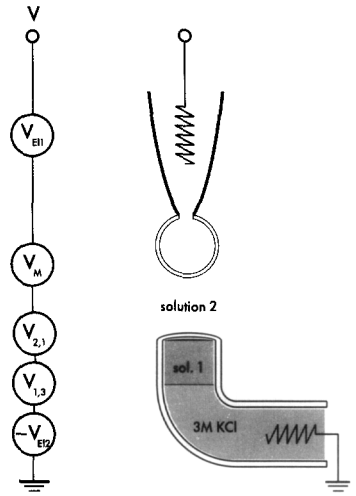

FIG. 3. A nonoptimal salt bridge. The right-hand side schematically shows a salt bridge which is initially filled with KCl–agar. When the experiment proceeds for an extended time using predominantly Ringer's solution in the bath, the terminal portion of the agar bridge will slowly equilibrate with Ringer's solution (solution 1). If then, for a short time, the bath solution is switched to solution 2, there will be effectively a double-bridge arrangement with an interface solution 2–solution 1 between the salt bridge and the bath, and an interface solution 1–KCl in the interior of the salt bridge. The associated liquid junction potentials are indicated schematically at left.

Measurement of Liquid Junction Potentials

Accurate measurement of liquid junction potentials requires a salt bridge with an abrupt boundary between the 3 *M* KCl solution and the test solution (see above). Such a device is readily made from a wide-bore patch pipette (~2 μm diameter) filled with 3 *M* KCl. This pipette, with a well-chlorided silver wire inside, is used as the bath ground. Another patch pipette is filled with the solution to be tested, and mounted onto the patch clamp headstage.

Three conditions have to be fulfilled for an accurate measurement: (1) The salt bridge should be the only ground connection of the bath, that is, any liquid leaks to grounded parts of the setup have to be avoided. This point is easily checked by temporarily removing the salt bridge from the bath. The patch-clamp amplifier should then read zero current, usually with large noise and movement artefacts superimposed. (2) The electrode potentials should be stable, that is, no drift in zero-current potential should occur when the bath solution is stationary (< 1 mV in 10 min). If this is not the case, then the silver wires need to be rechlorided. (3) Both patch pipettes should be filled sufficiently to guarantee a small steady outflow of

TABLE I
LIQUID JUNCTION POTENTIALS V_{LJ} OF STANDARD SALINE WITH RESPECT TO EXEMPLARY SOLUTIONS[a]

Solution	Composition[b]	V_{LJ} (mV)
Low sodium Ringer's	32 NaCl, 108 Tris-Cl, 2.8 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 10 NaOH–HEPES	−3
Sulfate Ringer's	70 Na_2SO_4, 70 sorbitol, 2.8 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 10 NaOH–HEPES	+6
Glutamate internal solution	145 Potassium glutamate, 8 NaCl, 1 $MgCl_2$, 0.5 ATP, 10 NaOH–HEPES	+10
Chloride internal solution	145 KCl, 8 NaCl, 1 $MgCl_2$, 0.5 ATP, 10 NaOH–HEPES	+3
Cesium glutamate internal solution	145 Cesium glutamate, 8 NaCl, 1 $MgCl_2$, 0.5 ATP, 10 NaOH–HEPES	+11
Cesium citrate internal solution	60 Cesium citrate, 10 CsCl, 8 NaCl, 1 $MgCl_2$, 0.5 MgATP, 20 CsOH–HEPES	+12

[a] Composition of saline (numbers in m*M*): 140 NaCl, 2.8 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 10 NaOH–HEPES.
[b] Values given as millimolar; for convention on polarity see the example on p. 127.

solution at the tip, otherwise the "double-liquid junction effect" previously discussed will result.

To start the measurement, the bath is first filled with the test solution (which also is in the pipette). The instrument is switched to current clamp mode, and the voltage reading on the amplifier is set to zero by adjusting the offset. The bath fluid is then exchanged for Ringer's solution (or other reference solution). The voltage reading should rapidly approach a stable value, which is the desired liquid junction potential with the polarity opposite to that required for use in Eqs. (1)–(7) (because the convention considers bath potential with respect to pipette). Following this the bath solution should be switched back to the test solution to check for reversibility.

Values of several test solutions against Ringer's solution are given in Table I. They range from −5 to +12 mV. The predominant salt of the test solution sets the value; minor additions of other salts and buffers usually have negligible effects. More values for liquid junction potentials are given in Ref. 3, together with equations and values to calculate V_{LJ} theoretically.

Summary

This chapter describes corrections that have to be applied to measured membrane potentials in patch clamp experiments. Some of them [Eqs. (1)–(3)] are required regardless of the nature of the reference electrode (in the Ringer's solution bath) whenever the pipette-filling solution is different

from the bath solution. They represent the liquid junction potentials that are present at the pipette tip before patch formation. In addition, corrections have to be applied when the bath solution is being changed during a measurement (i.e., after seal formation). In that case the following rules apply. (1) The new solution should never get into contact with the bare silver/silver chloride wire of the reference electrode. This requirement is best met by using a salt bridge. (2) The "best" salt bridge is a 3 *M* KCl bridge with an abrupt KCl–bath fluid boundary at its tip (see above). This bridge does not require any additional potential corrections, but it may lead to KCl poisoning of the bath or become contaminated by solutions used previously. (3) Local solution changes (microperfusion by puffer pipette, U tool or sewer pipe arrangements) as well as recessed KCl bridges require additional corrections, which (together with the simple liquid junction potential correction) are approximately given by Eqs. (6)–(8).

It should be stressed that all equations given here represent approximate corrections, since liquid junction potentials are thermodynamically ill-defined. This is particularly relevant for Eqs. (6) and (7) where the sum of two liquid junction potentials appears.

[7] Nonstationary Noise Analysis and Application to Patch Clamp Recordings

By STEFAN H. HEINEMANN and FRANCO CONTI

Introduction

Noise analysis was the first type of measurement that yielded reliable quantitative estimates of single-channel parameters[1-3] and thereby provided evidence for the very existence of channel proteins embedded in the lipid matrix of excitable membranes. It is still a powerful tool for investigation of ion transport mediated by channels.

The more direct way of measuring single-channel properties is the recording of unitary events using the patch clamp technique.[4,5] Single-channel recordings provide the richest information on the kinetics of the

[1] B. Katz and R. Miledi, *Nature (London)* **226,** 692 (1970).
[2] F. Conti, L. J. DeFelice, and E. Wanke, *J. Physiol. (London)* **248,** 45 (1975).
[3] E. Neher and C. F. Stevens, *Annu. Rev. Biophys. Bioeng.* **6,** 345 (1977).
[4] E. Neher and B. Sakmann, *Nature (London)* **260,** 799 (1976).
[5] O. P. Hamill, A. Marty, E. Neher, B. Sakmann, and F. J. Sigworth, *Pfluegers Arch.* **391,** 85 (1981).