A unified procedure for the correction of liquid junction potentials in patch clamp experiments on endo- and plasma membranes

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Received 6 August 1996; Accepted 22 October 1996

Abstract

Correct determination of absolute values and polarities of liquid junction potentials (LJPs) is essential in patch clamp experiments for the estimation of ionic selectivities and activation voltages of ion channels.

A simple approach for the correction of membrane voltages for LJPs has been developed. The method covers all combinations of LJPs between the solutions of bath, patch pipette and reference salt bridge and is applicable to all patch configurations on plasma membranes and endomembranes.

Key words: Patch clamp, liquid junction potentials, salt bridges, membranes, ion channels.

Introduction

There is hardly any patch clamp circuit which does not include liquid junction potentials (LJPs) arising at the interface between different salt solutions due to the different mobility of ions. For solutions of general use in electrophysiological experiments values of LJPs are in the range of 1 to 20 mV. Misestimation of the membrane voltage by only a few millivolts due to neglect or erroneous correction of LJPs may have a strong impact on the determination of selectivity coefficients of ion channels.

Several publications have dealt in detail with the questions of how to calculate and measure LJPs (Barry, 1989; Barry and Lynch, 1991; Neher, 1992). These papers provide a sound theoretical basis for such calculations. In everyday laboratory conditions, however, several factors may complicate the straightforward determination of the appropriate correction for a specific experimental situation.

One such factor relates to the wide range of experimental conditions used in patch clamp to answer certain questions. Each specific combination of solutions in patch pipette, reference salt bridge and bath creates LJPs at different sites of the circuit and at different times during the experiment. Furthermore, the functional polarity of the membrane patch will vary according to its origin, i.e. plasma membrane or endomembrane, and the patch can be orientated in different ways in the suction pipette exposing its cytosolic or extracytosolic side to the bath. A uniform polarity convention for membrane voltages across plasma membrane and endomembranes has been introduced (Bertl et al., 1992; Azzone et al., 1993) and equations for the correction of LJPs should be standardized accordingly.

Recently, software has been designed which displays the appropriate corrections for different patch configurations on the plasma membrane (Barry, 1994). Both plasma and endomembranes are considered here and a unified and accurate procedure for the correction of membrane voltages for LJPs is presented. The method takes account of all combinations of LJPs arising at the patch pipette and the reference salt bridge and of all patch configurations. Conventions set up by other authors have been maintained in order to avoid new definitions and it is hoped that this approach helps to standardize the treatment of LJPs in patch clamp experiments and to avoid erroneous interpretation of patch clamp data due to neglect of certain LJPs or to incorrect calculation of polarities.

Polarity conventions

Table 1 assigns the polarities of potentials used in the following equations. In accord with previous papers

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Table 1. Definitions and polarities of potentials in the patch clamp circuit

<table>
<thead>
<tr>
<th>Potential</th>
<th>Taken as</th>
<th>With respect to</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_{\text{pip}}$</td>
<td>Pre-sealing bath solution</td>
<td>Pipette solution*</td>
</tr>
<tr>
<td>$E_{\text{ref}}$</td>
<td>Pre-sealing bath solution</td>
<td>Reference solution*</td>
</tr>
<tr>
<td>$E_{\text{ef}}$</td>
<td>Post-sealing bath solution</td>
<td>Reference solution*</td>
</tr>
<tr>
<td>$V_{\text{hold}}$</td>
<td>Patch electrode</td>
<td>Reference electrode*</td>
</tr>
<tr>
<td>$E_{\text{offset}}$</td>
<td>Patch electrode</td>
<td>Reference electrode*</td>
</tr>
<tr>
<td>$E_{\text{m}}^c$</td>
<td>Cytosolic side*</td>
<td>Extracytosolic side*</td>
</tr>
<tr>
<td>$E_{\text{m}}^e$</td>
<td>Cytosolic side*</td>
<td>Extracytosolic side*</td>
</tr>
</tbody>
</table>

a According to Barry (1989).

b As set by the amplifier.

c See Bertl et al. (1992) and Table 1 in Azzone et al. (1993) with 'cytosolic' being equivalent to 'In' and 'extracytosolic' being equivalent to 'Out'.
d Potential of the cell or the compartment in attached patch configurations.
e Membrane potential.

(Barry, 1989; Barry and Lynch, 1991; Neher, 1992) all LIPS are taken as the potentials of the bath solution with respect to the pipette solution. Voltages which are set by the amplifier are used with the same polarity as they are displayed by a standard amplifier (potential of the patch electrode with respect to reference electrode). Membrane voltages have traditionally been expressed as cytosolic potentials with respect to extracytosolic potentials and this convention has been expanded to endomembranes (Bertl et al., 1992; Azzone et al., 1993). This general terminology for the definition of the polarity of different patch configurations is adopted by using the expressions cytosolic-side out (co) and cytosolic-side in (ci) patches rather than inside-out and outside-out patches. (For the inner membranes of mitochondria and chloroplast envelopes, as well as the thylakoid membranes, the expression 'cytosolic-side' is homologous to the definition of the operational 'in-side' proposed by Azzone et al., 1993.) Table 2 classifies the various patch clamp configurations with respect to this convention.

Table 2. Classification of different experimental patch configurations

<table>
<thead>
<tr>
<th>Membrane</th>
<th>Experimental patch configuration</th>
<th>General expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma membrane*</td>
<td>Inside out</td>
<td>Cytosolic-side out</td>
</tr>
<tr>
<td></td>
<td>cell attached</td>
<td>(co)</td>
</tr>
<tr>
<td></td>
<td>Outside out whole vacuole</td>
<td></td>
</tr>
<tr>
<td>Tonoplast†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma membrane*</td>
<td>Outside out whole cell</td>
<td>Cytosolic-side in</td>
</tr>
<tr>
<td></td>
<td>cell attached</td>
<td>(ci)</td>
</tr>
<tr>
<td></td>
<td>Inside out vacuole attached</td>
<td></td>
</tr>
<tr>
<td>Tonoplast†</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Similarly: inner membrane of mitochondria and chloroplast envelopes.
† Similarly: endoplasmic reticulum, outer membrane of mitochondria and chloroplast envelopes, thylakoid membrane.

Pre-sealing situation

Figure 1 shows the set-up for a general case of zero-current adjustment prior to the formation of a membrane seal. Both electrodes are linked via salt bridges (the pipette solution P and the reference solution R) to the bath (B°) with LIPS, $E_{\text{pip}}^0$ and $E_{\text{ref}}^0$, arising at the interfaces between the solutions. (Potential differences between the Ag/AgCl wires are not dealt with explicitly here, since they can be compensated before the start of an experiment and do not change during it.) The amplifier (A) provides settings for a holding potential ($V_{\text{hold}}$) and an offset ($E_{\text{offset}}$). During adjustment of the zero current $V_{\text{hold}}$ is set to zero. In a closed Kirchhoff loop all potentials add up to zero. Thus, traversing the loop in an anti-clockwise direction, results in:

$$E_{\text{offset}} + E_{\text{pip}}^0 - E_{\text{ref}}^0 = 0$$

$E_{\text{offset}}$ which had been defined with the opposite polarity (Table 1), appears with a negative sign. In practical terms, this means that after adjustment of the zero-current the amplifier provides a voltage offset which includes the electrode potentials (not explicitly treated here, see above) and the LIPS.

$$E_{\text{offset}} = E_{\text{ref}}^0 - E_{\text{pip}}^0$$

Fig. 1. General patch-clamp circuit prior to the formation of a seal. Open bars represent potentials set by the amplifier, black bars represent LIPS. The amplifier (A) provides settings for the holding potential ($V_{\text{hold}}$) and an offset voltage ($E_{\text{offset}}$). $V_{\text{hold}}$ is set to zero. LIPS, $E_{\text{pip}}^0$ and $E_{\text{ref}}^0$, arise at the interfaces between the solution in the patch pipette (P) and the bath solution (B°), and between the reference salt bridge (R) and the bath solution, respectively. Arrows indicate the polarity of the potentials (depicted as head of the arrow minus tail of the arrow). According to Kirchhoff's law all potentials in the closed loop sum to zero.
This voltage offset will be added to the holding potential throughout the whole experiment although the number and magnitudes of LJP s in the circuit change. (Some experimenters prefer to reset the offset before sealing by subtracting the calculated or measured value for $E_{\text{offset}} = E_{\text{pip}}^0$, in which case the remaining offset includes only the electrode potentials. In this situation the post-sealing equations (see below) will apply with $E_{\text{offset}}$ taken as equal to zero.)

**Examples**

3 M KCl in the reference bridge: Reference bridges with very high concentrations of KCl are frequently used to minimize the LJP between the bridge and the bath and render it independent of the bath solution. Equation 2 would therefore simplify to $E_{\text{offset}} = -E_{\text{pip}}^0$. However, this is only true for a standard situation in which the bath solution is composed mainly of ions with similar mobility at 100–200 mM concentration (resulting in a LJP of about −2 mV). The LJP may become more substantial when ions with lower mobility (such as Ca$^{2+}$) dominate the bath. Another drawback of 3 M KCl bridges is that Cl$^-$ potentials at the Ag/AgCl wires produce high voltage offsets that may be difficult to compensate. Furthermore, as pointed out by Barry (1989) and Neher (1992), even the positive features of 3 M KCl bridges mentioned above depend on the bridge being fresh or free-flowing.

Some solutions in reference bridge and bath before sealing: This is a good approach because it minimizes any history-dependent effects if the bath solution is going to be changed (Barry and Diamond, 1970). With $E_{\text{ref}}^0 = 0$, equation 2 simplifies to $E_{\text{offset}} = -E_{\text{pip}}^0$.

Some solutions in patch pipette and reference bridge: This approach retains a simple description of the LJP s and circumvents the disadvantages associated with 3 M KCl bridges. However, its use is restricted to situations in which the pipette solution has no special requirements (such as high CaCl$_2$, channel blockers or F$^-$ ions). Hence with $E_{\text{ref}}^0 = E_{\text{pip}}^0$, it follows that $E_{\text{offset}} = 0$.

**Post-sealing situation**

After establishing a membrane seal at the patch pipette, $E_{\text{pip}}^0$ is replaced by the membrane voltage over the patch, $V_m$. $V_m$ should ideally be set by the holding potential, $V_{\text{hold}}$, but its actual value depends on all other potentials in the loop. These are: (i) $E_{\text{offset}}$, (ii) $E_{\text{ref}}^0$, the LJP at the reference bridge, and (iii) $E_c$, the membrane voltage of the cell or the compartment in cases where attached patches are used. Figure 2 shows a general post-sealing circuit for attached patches. In the case of whole cell, whole compartment or excised patch configurations $E_c$ is equal to zero. Again all potentials taken in the closed loop sum to zero. Every other patch situation is a simplification of the one shown here. For excised and whole-cell (-compartment) patches $E_c$ is equal to zero. The polarity of $V_m$ depends on the membrane type and the patch configuration.

Kirchhoff loop sum to zero, so that

$$\pm V_m + V_{\text{hold}} + E_{\text{offset}} - E_{\text{ref}}^i + E_c = 0 \quad (3)$$

The signs of the potentials arise from their polarity defined in Table 1 and their position in the loop. The sign of $V_m$ depends on the patch configuration (Table 2) and both it and the sign of $E_c$ depend on the type of membrane. The general post-sealing equations are then:

$$\begin{align*}
\text{(co)} V_m & = -V_{\text{hold}} - E_{\text{offset}} + E_{\text{ref}}^i + E_c \\
\text{(ci)} V_m & = V_{\text{hold}} + E_{\text{offset}} - E_{\text{ref}}^i + E_c
\end{align*} \quad (4a,b)$$

**Examples**

Whole vacuole configuration; same solutions in patch pipette and reference bridge; bath solution initially B$^0$ and subsequently changed to B$^1$ and B$^2$: The whole vacuole configuration is a co-configuration (Table 2) and hence equation 4a should be used. $E_{\text{offset}} = 0$ according to equation (2) and $E_c = 0$ since no vacuole-attached patch is involved. The LJP s between the reference bridge and the bath can change if the bath is changed ($E_{\text{ref}}^i$ for every bath B$^i$). Again all potentials in the closed loop sum to zero. Every other patch situation is a simplification of the one shown here. For excised and whole-cell (-compartment) patches $E_c$ is equal to zero. The polarity of $V_m$ depends on the membrane type and the patch configuration.

$$\begin{align*}
V_m + V_{\text{hold}} + E_{\text{offset}} - E_{\text{ref}}^i - E_c &= 0 \\
\text{Fig. 2. Post-sealing patch-clamp circuit for an attached patch. In addition to the potentials defined for Fig. 1 the circuit includes the membrane voltage $V_m$ across the patch and the potential of the cell or compartment (C) $E_c$ (hatched bars). The LJP between the reference bridge and the bath can change if the bath is changed ($E_{\text{ref}}^i$ for every bath B$^i$). Again all potentials in the closed loop sum to zero. Every other patch situation is a simplification of the one shown here. For excised and whole-cell (-compartment) patches $E_c$ is equal to zero. The polarity of $V_m$ depends on the membrane type and the patch configuration.}
\end{align*}$$
Outside out patch configuration on plasma membrane; reference bridge contains pre-sealing bath solution $B^O$; bath solution initially $B^O$ and then changed to $B^I$. The patch configuration is $ci$ (Table 2) and equation 4b therefore applies. $E_{\text{offset}} = -E_{\text{pip}}^0$ according to equation 2, $E_{c}=0$ since the patch is not cell-attached, and $E_{\text{ref}}^i$ has to be determined for the new bath $B^I$. The following corrections result: $V_m = V_{\text{hold}} - E_{\text{pip}}^0$ (for bath $B^O$), and $V_m = V_{\text{hold}} + E_{\text{pip}}^i - E_{\text{ref}}^i$ (for bath $B^I$).

### Special cases

**Whole cell patching of large cells:** In large cells the cytosol may not completely exchange with the pipette solution and may produce an additional LJP, $E_{\text{pip}}^i$, between the pipette and the cytosol. (As pointed out by Barry and Lynch (1991) and Neher (1992) there is generally a Donnan-type contribution to $E_{\text{pip}}^i$ due to the presence of large fairly immobile organic anions in the cells.) In this case an additional term has to be introduced: $V_m = V_{\text{hold}} + E_{\text{offset}} + E_{\text{pip}}^i$. The exact amount of $E_{\text{pip}}^i$ is difficult to determine but considering its polarity may help to estimate the error.

**Local perfusion:** Selective perfusion of the bath in the local environment of the cell or membrane patch introduces an additional LJP between the new and the old bath solution into the patch circuit (Neher, 1992). Care has to be taken to add the term with the right polarity since the convention for bath and pipette solution is now meaningless. Logically, this case is equivalent to changing the bath solution with the reference bridge composition being the same as that of the original bath. The LJP between the two solutions can be determined according to the methods described below with the original bath solution in the pipette (Method 1) or in the reference bridge (Method 2) and the new solution in the bath. The resultant LJP should then be added to the right side of equations 4a and b with the same sign as $E_{\text{ref}}^i$.

### Measuring LJPs

#### Method 1

By using a fresh 3 M KCl reference bridge LJPs between different bath solutions and different pipette solutions (including the reference solution used in the experiment) can be determined as explained by Neher (1992). A voltage offset due to electrode potentials is zereod with identical solutions in the pipette and the bath. The bath is subsequently exchanged with the experimental bath solutions in question. The polarity of the potentials so measured is always opposite to the defined polarity of LJPs (Table 1) and has therefore to be multiplied by $-1$.

#### Method 2

An alternative method for the determination of LJPs simulates a patch configuration. Thus, $E_{\text{pip}}^0$ is set to zero with identical solutions in pipette and bath ($B^0$). The reference bridge with reference solution is positioned in a second bath ($B^1$) linked to the first via a fresh 3 M KCl bridge. After compensation of electrode potentials with reference solution in $B^1$, LJPs between the reference bridge and the bath (as they arise after seal formation) can be measured by perfusion of $B^1$ with experimental solutions. The polarity of the measured potentials is then identical to the definitions in Table 1.

### Application

A rapid procedure for obtaining the appropriate LJP correction in a specific experimental situation can be achieved according to the following steps.

Step 1: Measure (as above) or calculate LJPs of the solutions in use and determine their sign according to the convention given in Table 1.

Step 2: Specify the pre-sealing situation and determine the appropriate $E_{\text{offset}}$ according to equation 2.

Step 3: Classify the patch configuration according to Table 2.

Step 4: Specify the post-sealing situation(s) and choose the appropriate equation (equations 4a, b).

Step 5: Insert the values for $E_{\text{offset}}$ and $E_{\text{ref}}^i$ into equations 4a and b and determine the corrected value for $V_m$.

### Acknowledgements

We would especially like to thank Professor Peter Barry for his critical reading of the manuscript and helpful comments. The work was funded by the European Communities’ BIOTECH programme, as part of the Project of Technological Priority 1993–1996.

### References


