

Editorial

What is the true resting potential of small cells?

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In order to understand almost anything, it is necessary to first obtain a measurement of the phenomenon which is not altered by experimental artifacts. This fundamental principle is particularly true for the determination of cell resting potential which not only controls the electrical activity of excitable cells (see Wilson and Kawaguchi 1996) and the free intracellular Ca^{2+} concentration (see Kamouchi et al. 1999) but is thought to control various cellular functions such as apoptosis and proliferation (Ghiani et al. 1999; Wang et al. 1999).

It is now clear that the potential across the membrane of a cell is, at any instant, a direct consequence of the various ionic conductances and pumps or exchangers, with asymmetric ionic stoichiometries, which are present in the membrane and are active at that time. In addition to the membrane's own ionic mechanisms, attempts to directly measure the membrane potential and conductance almost inevitably introduce another conductance (also called the shunt) which will influence to various degrees what is taken to be the potential of the cell.

In giant axons, the membrane potential can be recorded between an external electrode and an electrode axially introduced into the axoplasm. In thin nerve and muscle fibres, the membrane potential can be recorded between an external electrode and an electrode located near an electrically isolated cut end of the fibre bathed with a solution producing no liquid junction potential with the cytoplasm. In these cases, the shunt resistance is sufficiently greater than that of the membrane and has a negligible effect.

In general, cells are too small to apply the above techniques, and the membrane potential is measured between an electrode in the extracellular solution and a thin microelectrode introduced into the cytoplasm through the plasma membrane. However, membrane potentials measured in this way may be disturbed by the low electrical resistance around the penetrating electrode. The membrane potential may also be measured electrically with the whole-cell or the perforated patch-clamp methods used in the zero current or the current clamp mode. In this case, the shunt resistance can be very much greater than with an impaling microelectrode. However, with very small cells or cells with membrane resistance in the order of $\text{G}\Omega$, changes in the seal resistance (when changing from the cell-attached to the whole-cell or the perforated configuration) will alter the measured potential, which may also be disturbed by alterations of intracellular ion composition *via* the pipette

solution. Moreover, in both of the above techniques, the membrane potential of cells with high membrane resistance can be influenced by junction potentials between the recording electrode and the cytoplasm or by small offset currents delivered by the recording amplifier or the electrical stimulator.

Another method to determine the membrane potential of small cells is to measure the equilibrium distribution of voltage sensitive dyes between the extra- and intracellular phases of the membrane. However, membrane potentials measured with such an optical method can be altered if the dye is not equally distributed in the cytoplasm or accumulates in cell organelles. Moreover, membrane resistance measurements which require the injection into the cell of a depolarizing or a hyperpolarizing current cannot be made with this method. For these reasons, optical methods are less used than electrical ones.

In view of the above possible artifacts, I would like to raise some experimental and theoretical contradictions arising from resting potential determinations in neuroblastoma and glioma cells, which are classical models for normal and tumoral brain cells.

Since the Bernstein hypothesis, the resting potential of animal cells is universally assumed to be mainly determined by passive permeability of the membrane for K^+ ions (i.e. K^+ channels). If one neglects Na^+ and Cl^- permeabilities of the membrane, the resting potential equals the Nernst potential for K^+ ions. Such is the case for skeletal muscles and some neurons and glia cells, the resting potential of which is very close to the equilibrium potential for K^+ ions. For most other cells, less negative resting potentials are assumed to arise from a non negligible permeability for Na^+ and/or Cl^- ions, with more positive equilibrium potentials. If this conclusion is correct, for several cell types less negative resting potentials can also be due to the fact that the shunt resistance around the recording electrode, with a zero equilibrium potential, contributes to the measured potential. This may explain how the resting potential, measured under the same experimental conditions and in similar cells, may vary according to experiments and methods used, between -30 mV and -90 mV (see Lichtshtein et al. 1979; Brismar 1995; Bianchi et al. 1998). Moreover, the cell input resistance measured in the same cell types with a microelectrode is generally much smaller (in the order of $10^7 \Omega$) than when measured with a patch-clamp pipette (in the order of $10^9 \Omega$). From these examples it is clear that the measured resting potential of small cells with low ion channel densities (i.e. with a high membrane resistance) is largely dependent on the shunt resistance around the recording electrode (see Fig. 1A), in accordance with Ohm's law:

$$\frac{1}{R_{in}} = \frac{1}{R_m} + \frac{1}{R_s}, \quad (1)$$

where: R_{in} = input resistance, R_m = membrane resistance and R_s = shunt resistance.

In general, resting potentials of neuroblastoma and glioma cells are almost similar when measured with the patch-clamp method or with dyes but are less negative when measured with a microelectrode. Moreover, potentials are more negative as

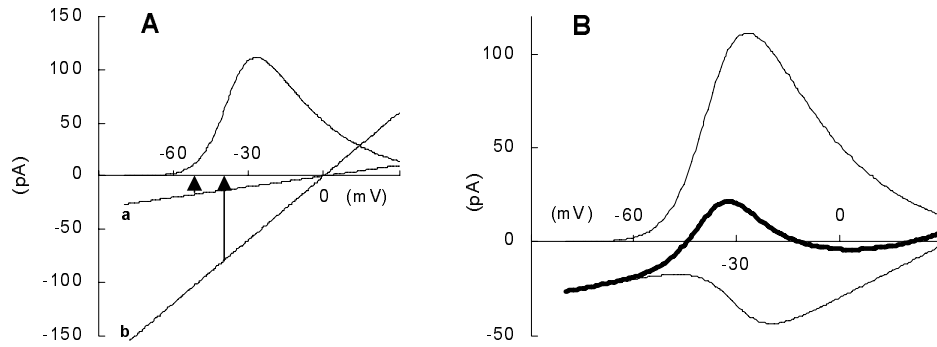


Figure 1. Membrane current-voltage curves and zero current potentials.

Theoretical steady-state current-voltage curves and zero current potentials (resting potentials) of a cell which possesses voltage dependent K^+ and Na^+ channels and voltage independent non selective channels.

A. The specific Na^+ current was ignored. The steady-state outward K^+ current, calculated from values reported by Arcangeli et al. (1995) for a HERG-like current is the product of g (activation) \times h (inactivation) \times $g_{max} \times (V - V_K)$. Steady-state activation and inactivation were calculated from the Boltzman equation:

$$g \text{ or } h = 1/(1 + \exp((V - V_{0.5})/k)) \quad (2)$$

with $V_{0.5} = -49$ mV (activation) and -37 mV (inactivation) and $k = 18$ mV (activation) and -5.5 mV (inactivation). $g_{max} = 10$ nS and $V_K = -84$ mV. The non specific current was assumed to reverse at 0 mV membrane potential. Between -70 mV and -80 mV, the input resistance is 3 G Ω (a) and 0.5 G Ω (b). The zero current voltage (arrows) is -49.5 mV (a) and -37.5 mV (b). According to Eq. (1), assuming that this difference is due to a change in the shunt resistance (R_s) and if R_s equals 10 G Ω in (a), it is only 0.566 G Ω in (b), the membrane resistance (R_m) between -70 mV and -80 mV equals 4.286 G Ω and the true resting potential (with an infinite R_s) is -51.5 mV.

B. Same conditions that in (Aa) but with an additional persistent Na^+ current which is the product of g (activation) \times $g_{max} \times (V - V_{Na})$. The steady-state activation was calculated from the Boltzman equation with $V_{0.5} = -30$ mV and $k = -5$ mV. $g_{max} = 1.2$ nS and $V_{Na} = 50$ mV. The thick curve is the total current. It has three reversal potentials which correspond to three possible resting potentials.

the input resistance is larger. For instance, in patch-clamped human neuroblastoma cells, Ginsborg et al. (1991) measured resting potentials and input resistances between -30 mV and -60 mV and 0.8 G Ω and 3 G Ω respectively. This scattering can have at least three explanations, namely: 1) the membrane conductance and the ratio of K^+/Na^+ conductances vary from one cell to another (see McKhann et al. 1997, for resting membrane potential values reported for astrocytes); 2) the smaller values of the input resistance are due to a low seal resistance between the pipette and the membrane; 3) an inward depolarizing-activated current contributes to the resting potential (see Fig. 1B). In this latter case, it must be noted that de-

pending on the active channel types, cells can have several zero current potentials and thus, under certain conditions (injection of a current into the cell, postsynaptic potentials or synchronized opening of several channels), the resting potential can switch from one value to another as already observed on nerve fibres and neurons (Stämpfli 1959; Wilson and Kawaguchi 1996; Gola et al. 1998). However, in these cases, if the shunt resistance is decreased, only one resting depolarized potential can be obtained.

Uncertainties in the true value of the resting potential can lead us to think that some conclusions about the physiological roles for the resting potential are wrong, and can explain some contradictions in the literature concerning these roles. For instance, it is now widely recognized that plasma membrane K^+ channels control the mitogenesis of normal and tumor cells (see Dubois and Rouzaire-Dubois 1993; Wonderlin and Strobl 1996). Among other hypotheses, it has been assumed that the inhibition of cell proliferation by K^+ channel blockers is due to membrane depolarization (Ghiani et al. 1999). This is in apparent contradiction with both the observation that cancer cells are less polarized than normal cells and the conclusion that a depolarized resting potential is required for unlimited tumor growth (Bianchi et al. 1998). Reasons for this contradiction may be that the proliferation of cancer cells is not controlled by membrane potential (Rouzaire-Dubois and Dubois 1998) and/or the error in the resting potential determination of cancer cells is larger than that of normal cells because the former ones have a lower ion channel density and consequently, their measured resting potential is more dependent on the shunt resistance than that of the latter.

In conclusion, one can say that the question: “what is the true resting potential of small cells?” remains open. Given that it is impossible to know the relative contribution of the shunt resistance to the input resistance, resting potential values depend on exclusion criteria used to define the limit of depolarization of the cells that are included for analysis. Since these criteria are not objective, they can be at the origin of contradictory conclusions on the value of the resting potential of a given cell type and on its physiological roles (see for instance: Brismar 1995; McKhann et al. 1997; Bianchi et al. 1998).

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