

About a new method to measure fractional Ca^{2+} currents through ligand-gated ion channels

Sergio Fucile and Francesca Grassi

Department of Physiology and Pharmacology "V. Erspamer," Sapienza University, 00185 Rome, Italy

The *Journal of General Physiology* recently published a paper (Elenes et al., 2009) describing a novel electrophysiological approach to measure the Ca^{2+} -carried current through ligand-gated channels. The method proposed is based on the assumption that all permeant ions compete for a single binding site, which controls permeation and is implemented by fitting single-channel data obtained at different extracellular Ca^{2+} concentrations ($[\text{Ca}^{2+}]_o$) to derive values for the fractional Ca^{2+} current, i.e., the percentage of the total current carried by Ca^{2+} ions.

The fractional Ca^{2+} current values obtained are not too different from some of those found for the rat NR1-NR2A NMDA receptor or the human adult acetylcholine receptor (AChR) using the fluorometric method proposed by Zhou and Neher (1993). In contrast, the values found for the fetal and adult mouse AChR (19.3 and 16.3%) are at odds with the values present in the literature (~ 2 and $\sim 4\%$, respectively) (Villarroel and Sakmann, 1996; Ragozzino et al., 1998; Fucile et al., 2006). In particular, the fractional Ca^{2+} current values for the mammalian fetal AChR calculated using different experimental approaches are all comprised between 2.0 and 3.4% (Decker and Dani, 1990; Vernino et al., 1994; Villarroel and Sakmann, 1996; Ragozzino et al., 1998; Fucile et al., 2006).

Of note, the discrepancy is such that either the values of Elenes et al. (2009) or the previously published values are wrong. Here, we point out some issues that may have influenced the estimates by Elenes et al. (2009).

The authors decompose the total current (i_{total}) flowing through the AChR channel as the sum of a lumped current representing Na^+ , K^+ , and Mg^{2+} ions ($i_{\text{Na}^+ - \text{Mg}^{2+}}$) plus the current carried by Ca^{2+} ions ($i_{\text{Ca}^{2+}}$). From the "one-site" model they derive equations for $i_{\text{Na}^+ - \text{Mg}^{2+}}$ and $i_{\text{Ca}^{2+}}$, copied here with their original numbering, for the reader's convenience:

$$i_{\text{Na}^+ - \text{Mg}^{2+}} = \gamma_{\text{Na}^+ - \text{Mg}^{2+}} \frac{K_{D\text{Ca}^{2+}}}{K_{D\text{Ca}^{2+}} + a\text{Ca}_o^{2+}} V \quad (3)$$

$$i_{\text{Ca}^{2+}} = \gamma_{\text{Ca}^{2+}} \frac{a\text{Ca}_o^{2+}}{K_{D\text{Ca}^{2+}} + a\text{Ca}_o^{2+}} V \quad (4)$$

Noticeably, current reversal potential has been omitted in both equations.

Inserting into Eq. 4 the values of $K_{D\text{Ca}^{2+}}$ derived by fitting, the authors conclude that with an external solution containing 150 mM NaCl plus 100 mM CaCl_2 , the inward current is "carried exclusively by Ca^{2+} ions." This is a central point in the paper, as it is a key prediction of the model and is used to show that the error introduced neglecting current reversal potential in Eqs. 3 and 4 is small.

This statement is not supported by experimental data presented in the paper. With 100 mM CaCl_2 plus 150 mM NaCl in the external solution, the reversal potential of ACh-evoked currents is +18 mV (Elenes et al., 2009). When Ca^{2+} ions completely replace monovalent ions in the external solution (and therefore are the only inward-flowing ions), the reversal potential of the ACh-evoked current (E_{ACh}) undergoes a hyperpolarizing shift (Bregestovski et al., 1979; Lewis, 1979; Adams et al., 1980; Villarroel and Sakmann, 1996). In particular, with 100 mM CaCl_2 but no monovalent ions in the external solution, E_{ACh} is -22.3 and -10 mV for fetal and adult mouse AChR, respectively (Villarroel and Sakmann, 1996). The large difference of E_{ACh} in the presence or absence of extracellular Na^+ ions indicates that they do contribute measurably to the inward current, even when $[\text{Ca}^{2+}]_o$ is 100 mM. Should a high $[\text{Ca}^{2+}]_o$ prevent the flux of Na^+ ions, the latter would not influence E_{ACh} any more than other impermeant ions.

Furthermore, to our knowledge, it has never been demonstrated experimentally (for instance, by means of Na^+ -sensitive fluorescent dyes) that Na^+ ions do not flow through AChR channels if $[\text{Ca}^{2+}]_o$ is high. Ca^{2+} ions pass through AChR channels, although the unitary channel conductance is reduced in a Ca^{2+} -dependent manner (Bregestovski et al., 1979; Lewis, 1979; Decker

Correspondence to Francesca Grassi: francesca.grassi@uniroma1.it
Abbreviation used in this paper: AChR, acetylcholine receptor.

and Dani, 1990; Grassi and Degasperis, 2000; Elenes et al., 2009) until it reaches a plateau value at $[Ca^{2+}]_o$ of ~ 40 mM. At high $[Ca^{2+}]_o$, unitary conductance of AChR channels is independent of the presence of monovalent cations, which has been interpreted as suggesting that at high $[Ca^{2+}]_o$, Ca^{2+} ions are the main current carriers (Decker and Dani, 1990).

Below, we show how including the reversal potential for $i_{Ca^{2+}}$ and $i_{Na^+-Mg^{2+}}$ ($E_{Ca^{2+}}$ and $E_{Na^+-Mg^{2+}}$, respectively) in Eqs. 3 and 4, and dropping the unsupported assumption that with $[Ca^{2+}]_o = 100$ mM Na^+ ions do not contribute to inward current, leads to conclusions that are very different from those presented by Elenes et al. (2009), yet compatible with their experimental data.

Eqs. 3 and 4 must be amended as follows:

$$i_{Na^+-Mg^{2+}} = \gamma_{Na^+-Mg^{2+}} \frac{K_{D_{Ca^{2+}}}}{K_{D_{Ca^{2+}}} + aCa_o^{2+}} (V - E_{Na^+-Mg^{2+}}) \quad (3a)$$

$$i_{Ca^{2+}} = \gamma_{Ca^{2+}} \frac{aCa_o^{2+}}{K_{D_{Ca^{2+}}} + aCa_o^{2+}} (V - E_{Ca^{2+}}) \quad (4a)$$

Under the experimental conditions used by Elenes et al. (2009) ($[Na^+]_o = 150$ mM and $[Ca^{2+}]_o = 100$ mM), $E_{Na^+-Mg^{2+}}$ is indeed close to 0 mV, but $i_{Ca^{2+}}$, being a pure Ca^{2+} current, changes sign at the Ca^{2+} equilibrium potential. $E_{Ca^{2+}}$ must not be confused with the bi-ionic reversal potential of a current resulting from the steady-state counter-diffusion of extracellular Ca^{2+} and

intracellular K^+ ions (such as that measured by the experiment shown by Elenes et al., 2009 in their Fig. 15), as the current carried by K^+ ions is included in the term $i_{Na^+-Mg^{2+}}$ in the present model. The slope conductance of $i_{Ca^{2+}}$ and $i_{Na^+-Mg^{2+}}$ can be calculated from Eqs. 3a and 4a (Fig. 1 A; see figure legend for details). Using numerical values given by Elenes et al. (2009), we find that i_{total} with a slope conductance of 30.6 pS corresponds to a pure $i_{Ca^{2+}}$ with a slope conductance of 7 pS. When the I-V curve is shifted to the left (as done by Elenes et al., 2009), the error introduced is much larger for i_{total} than for $i_{Ca^{2+}}$ because of their different conductances, thereby causing a marked overestimation of the ratio $i_{Ca^{2+}}/i_{total}$.

At variance with the original equations, Eqs. 3a and 4a also describe $i_{Ca^{2+}}$ and $i_{Na^+-Mg^{2+}}$ as compatible with the experimental observation that, at high $[Ca^{2+}]_o$, single-channel conductance of i_{total} varies little regardless of whether monovalent cations are added to external solutions (Decker and Dani, 1990) (Fig. 1 B).

In conclusion, neglecting equilibrium potentials in Eqs. 3 and 4 causes overestimation of the fractional Ca^{2+} current. We therefore invite the authors to repeat their fits using Eqs. 3a and 4a, as given here. This might lead to estimates for the fractional Ca^{2+} current in closer agreement with other reports. It might also disclose why the estimates proposed by Elenes et al. (2009) are better for receptors thought to have high fractional Ca^{2+} current than for those with low ones.

Edward N. Pugh Jr. served as editor.

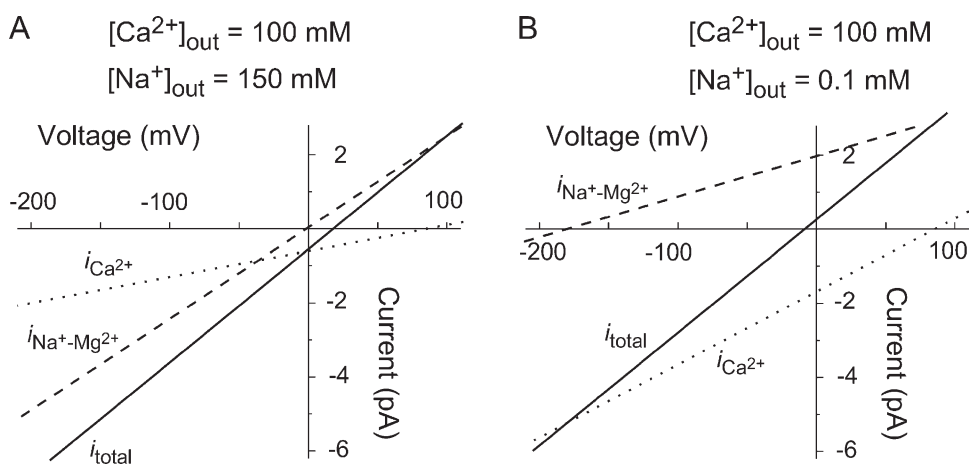


Figure 1. Decomposition of experimental I-V curves for the total current into the components carried by Ca^{2+} and by all other ions, assuming an ohmic behavior of all currents. In both A and B, $E_{Ca^{2+}} = 86$ mV, calculated using the Nernst equations with $[Ca^{2+}]_o = 100$ mM and $[Ca^{2+}]_i = 0.1$ mM (i.e., 0.1% of the main cation, a reasonable upper bound for the value). Total current (i_{total} ; solid lines) has a slope conductance of 30.6 pS (as in Fig. 15 of Elenes et al., 2009). (A) With $[Ca^{2+}]_o = 100$ mM and $[Na^+]_o = 150$ mM, i_{total} has a reversal potential of +18 mV and an amplitude of

-0.6 pA at 0 mV (Elenes et al., 2009). The pure Ca^{2+} current ($i_{Ca^{2+}}$; dotted line) is the line that passes through this experimental point and is 0 at $E_{Ca^{2+}}$, which yields a slope conductance of 7 pS. The lumped current carried by all other ions ($i_{Na^+-Mg^{2+}}$; dashed line) has been calculated by subtraction ($i_{total} - i_{Ca^{2+}}$) and reverts at 0 mV as expected. (B) When NaCl is not included into the external solution, we can estimate that $[Na^+]_o = 0.1$ mM and the Nernst equation yields $E_{Na^+-Mg^{2+}} = -180$ mV (neglecting Mg^{2+} for the sake of simplicity). We assumed that i_{total} has a reversal potential of -10 mV (Villarreal and Sakmann, 1996). $i_{Ca^{2+}}$ was calculated imposing $i_{Ca^{2+}} = i_{total}$ at -180 mV and $i_{Ca^{2+}} = 0$ at 86 mV, and has a slope conductance of 20 pS. $i_{Na^+-Mg^{2+}}$ reverts at $E_{Na^+-Mg^{2+}} = -180$ mV.

REFERENCES

- Adams, D.J., T.M. Dwyer, and B. Hille. 1980. The permeability of endplate channels to monovalent and divalent metal cations. *J. Gen. Physiol.* 75:493–510.
- Bregestovski, P.D., R. Miledi, and I. Parker. 1979. Calcium conductance of acetylcholine-induced endplate channels. *Nature.* 279:638–639.
- Decker, E.R., and J.A. Dani. 1990. Calcium permeability of the nicotinic acetylcholine receptor: the single-channel calcium influx is significant. *J. Neurosci.* 10:3413–3420.
- Elenes, S., M. Decker, G.D. Cymes, and C. Grosman. 2009. Decremental response to high-frequency trains of acetylcholine pulses but unaltered fractional Ca^{2+} currents in a panel of “slow-channel syndrome” nicotinic receptor mutants. *J. Gen. Physiol.* 133:151–169.
- Fucile, S., A. Sucapane, F. Grassi, F. Eusebi, and A.G. Engel. 2006. The human adult subtype ACh receptor channel has high Ca^{2+} permeability and predisposes to endplate Ca^{2+} overloading. *J. Physiol.* 573:35–43.
- Grassi, F., and V. Degasperi. 2000. Modulation of fetal and adult acetylcholine receptors by Ca^{2+} and Mg^{2+} at developing mouse end-plates. *Pflugers Arch.* 440:704–709.
- Lewis, C.A. 1979. Ion-concentration dependence of the reversal potential and the single channel conductance of ion channels at the frog neuromuscular junction. *J. Physiol.* 286:417–445.
- Ragozzino, D., B. Barabino, S. Fucile, and F. Eusebi. 1998. Ca^{2+} permeability of mouse and chick nicotinic acetylcholine receptors expressed in transiently transfected human cells. *J. Physiol.* 507:749–757.
- Vernino, S., M. Rogers, K.A. Radcliffe, and J.A. Dani. 1994. Quantitative measurement of calcium flux through muscle and neuronal nicotinic acetylcholine receptors. *J. Neurosci.* 14:5514–5524.
- Villarroel, A., and B. Sakmann. 1996. Calcium permeability increase of endplate channels in rat muscle during postnatal development. *J. Physiol.* 496:331–338.
- Zhou, Z., and E. Neher. 1993. Calcium permeability of nicotinic acetylcholine receptor channels in bovine adrenal chromaffin cells. *Pflugers Arch.* 425:511–517.