CFTR, investigated with the two-electrode voltage-clamp technique: the importance of knowing the series resistance

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Abstract

When the gene mutated in cystic fibrosis (CF) was identified and named the cystic fibrosis transmembrane conductance regulator (CFTR), it was not immediately clear what its function might be. Subsequent analysis showed that it is an anion channel, but also other functions were proposed. This protocol aims to shortly describe the two-electrode voltage-clamp (TEVC) technique and to highlight some precautions when studying CFTR with it. A short description of the series resistance ($R_s$) and its influence on measurements of channel characteristics is given.

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1. Introduction

The cystic fibrosis transmembrane conductance regulator (CFTR) was the first gene identified by positional cloning. Heterologous expression of CFTR and studies of endogenous CFTR firmly established that CFTR is an anion channel, which is regulated by protein phosphorylation and nucleotides. Other functions of CFTR were however also put forward, especially the regulation of “sodium absorption”.

The notion of increased sodium absorption has a long history in cystic fibrosis (CF) research, probably first proposed for the CF-sweat duct [1]. In 1983, it was suggested that sodium absorption is also increased in CF airways [2]. In line with these early observations, there appeared a patch-clamp study suggesting that CFTR inhibits the epithelial Na⁺ channel (ENaC) [3], raising the question whether CFTR has an additional function. Several studies on coexpressed CFTR and ENaC in oocytes have been published since 1995, suggesting that the activation of CFTR inhibits ENaC. We measured CFTR and ENaC currents in oocytes coexpressing them, and as we found no evidence for the interaction of CFTR with ENaC [4], suggested series resistance problems in previously reported experiments. Series resistance considerations are important not only in two-electrode voltage clamp (TEVC) but also in patch-clamp experiments (also called access resistance), see for example the illuminating review by Armstrong and Gilly [5]. This paper is a shortened version of an online published protocol [6] which aims to explain the series resistance problem and how it should be dealt with seriously.

2. Expression and electrical measurement of CFTR in oocytes

The methods to record from oocytes are described elsewhere (see online protocol [6] and references therein). After the injection of cRNA, oocytes are incubated for at least 24 h, usually up to 5 days, whereby expression increases with time. CFTR can easily be activated by elevating intracellular [cAMP], e.g., by application of forskolin and isobutylmethylxanthine (IBMX). As even low amounts of CFTR-mRNA (3 ng) induce a high membrane...
conductance (up to 0.5 mS), once activated, CFTR measurements require an amplifier with a high maximal voltage output (≥ 100 V) for injecting current.

3. Series resistance considerations in the voltage-clamping of oocytes

Simplified, we have an electrical circuit for measuring potential and one for injecting current, connected in a feedback amplifier. Although we are only interested in measuring the conductance of expressed proteins, we have to know the other relevant resistances in the electrical circuit, see Fig. 1 in online published protocol [6]. As these resistances are in series with the membrane resistance, they are each called series resistance ($R_s$). Obviously, we have an intracellular $R_s$ ($R_{si}$) between the impaled voltage electrode and the membrane and an extracellular $R_s$ ($R_{se}$) between the membrane and the bath voltage electrode. The extracellular $R_s$ is a sum of various components, depending on the experimental conditions, see Table 1. As the voltage circuit includes a high-resistance amplifier input, virtually no current is flowing in the electronic part of this circuit. Current is however flowing in the current circuit, injected by the impaled current electrode and returning via the bath current electrode. Therefore, current also flows in the ionic part of the voltage circuit. According to Ohm’s law, a voltage will drop along this pathway where the voltage across each resistance is the product of resistance and electrical current. Relevant resistances are the $R_{si}$, the membrane resistance, and the $R_{se}$—however, not the resistances of the voltage electrodes as virtually no current is passing through them. The $R_{si}$ seems to be low in the case of oocytes (probably less than 100 Ω). The $R_{se}$ in a Two-Bath-Electrode setup depends mainly on the distance of the bath voltage electrode from the oocyte. If this distance is kept small, $R_{se}$ may be in the range of 100 Ω. A larger distance however easily introduces an $R_s$ of several kΩ. The situation is even more serious when employing only one common bath electrode for the voltage and the current circuit. The $R_{se}$ in this One-Bath-Electrode setup not only depends on the distance of the bath electrode to the oocyte ($R_{seBa}$) but also on the resistance of the “agar bridge” or “flowing KCl bridge” ($R_{seBr}$), as well as on the resistance of the Ag/AgCl electrode ($R_{seAg}$), see Table 1. In practice, the $R_s$ then easily adds up to 5 kΩ or more. Below I will describe a simple method to estimate $R_{se}$. It is important to know $R_s$ for each experiment as it will influence the error in measuring the conductance of expressed proteins.

![Fig. 1. Demonstration of the series resistance dependence of apparent inhibition of ENaC by CFTR activation. Apparent ENaC conductances, determined with different series resistances from the same oocyte (with true conductances for CFTR of 200 μS and for ENaC of 100 μS), are shown. (a–d) Conductance measurements at different series resistances ($R_s$), as indicated. (1) Apparent ENaC conductance, i.e., conductance change, induced by activation of ENaC without CFTR activation. (2) Apparent ENaC conductance during activation of CFTR. At $R_s = 100 \, \Omega$, no inhibition of ENaC by CFTR activation is apparent. With increasing $R_s$, an inhibition of ENaC by activation of CFTR seems to appear.](image-url)
4. A series resistance may limit conductance increases

CFTR expresses very well in oocytes, i.e., activated CFTR "permeabilizes" oocytes efficiently for chloride. We obtained a CFTR-mediated conductance of 250 μS 2–3 days after injecting 3 ng of CFTR-mRNA [4]. To measure a conductance of 250 μS (4 kΩ) reliably, it is crucial that the $R_s$ does not exceed 200 Ω so that the measured conductance is not diminished by more than 5%. Is it possible to measure the $R_s$? The $R_s$ is equal to the total measured resistance if the membrane resistance is decreased to zero! Therefore, the $R_s$ can be easily obtained by measuring the resistance at zero membrane resistance, e.g., at the end of an experiment, simply by rupturing the oocyte membrane with the impaling electrodes or by pulling the electrodes out of the oocyte, still remaining in voltage-clamp mode.

Fig. 1 shows for a realistic example of an oocyte with a true CFTR conductance of 200 μS and a true ENaC conductance of 100 μS, how activation of CFTR does not significantly change the measured ENaC conductance at an $R_s$ of 100 Ω (Fig. 1a), whereas at higher $R_s$, the measured ENaC conductance appears smaller and seems to be "inhibited" by the activation of the CFTR conductance (Fig. 1b–d). Previous reports of reduced apparent inhibition of ENaC conductance by a weakly expressing CFTR mutant like F508del or a reduced chloride concentration, conditions which reduce CFTR conductance, may also be explained by measurements with a sizeable $R_s$.

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References