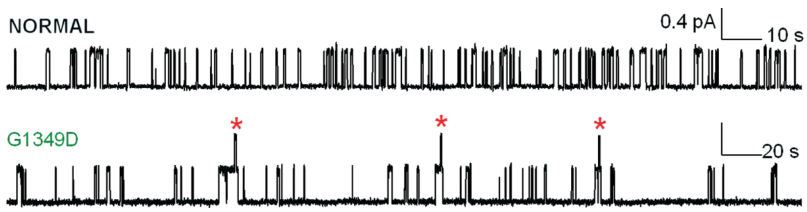
Chapter 20

Web Text Box 1

An electrophysiological study of a CFTR mutant



Electrophysiological record

In these records by Paola Vergani and David Gadsby we see single CFTR channels switching between the closed and open states.

In the top record, the electrical current sent through a patch of membrane containing a single normal CFTR is recorded. Every time the channel opens, the trace jumps up to a new steady level as chloride ions flow through the CFTR channel, carrying current.

In this experiment, the endogenous cytosol was replaced with an artificial solution containing 4 mmoles liter^{-1} chloride, while the extracellular medium contained 140 mmoles liter^{-1} chloride. This meant that there was a large concentration gradient for chloride directed inward towards the cytosol. To increase the electrochemical gradient further the experimenters set the membrane voltage to +40 mV so that the negatively charged chloride ions would be attracted inwards. They did this so that the change in current seen when the channel opened would be large enough, compared to the “noise” on the trace, to detect the opening event unambiguously. The artificial cytosol solution contained 5 mM ATP and 300 nM protein kinase A. The protein kinase A uses the ATP to phosphorylate the CFTR, keeping it in the active state.

The bottom record shows the behavior of a missense mutant in which a glycine at amino acid position 1349 has been replaced by an aspartate. When the channel is open, it passes the same size current as the normal channel. However, on average it spends much less time open (notice that the record represents twice as much time as the upper record). This is therefore a class III mutant (book page 333).

In fact the second patch of membrane contained at least two mutant channels (but likely more than that), because occasionally, as indicated by the red asterisks, the current jumps to a value twice that carried by a single CFTR, indicating that two channels are open simultaneously. In contrast, the patch of membrane that gave the top trace probably contained only one channel, since we never see such “double openings” (For the statistical arguments, scroll down to Text Box 20.1a, An electrophysiological study of a CFTR mutant: statistical arguments.)

Students who have studied electrophysiology should make sure that they understand why an inward movement of chloride ions appears as an upward deflection of the current trace in this figure. Once you think that you can explain why the deflections are upward, scroll down for our explanation in Web Text Box 20.1b, An electrophysiological study of a CFTR mutant: answer to the electrophysiological question.

Paola Vergani is in the Department of Neuroscience, Physiology and Pharmacology, University College London < http://www.ucl.ac.uk/npp/pv.html >.

David Gadsby is in the Laboratory of Cardiac/Membrane Physiology, Rockefeller University < http://www.rockefeller.edu/labheads/gadsby/gadsby_home.html >.

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Web Text Box 1.a

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STATISTICAL ARGUMENTS

Knowing (i) the cumulative time spent at the highest conductance level (level N) during the recording and (ii) the channel opening rate, calculated assuming the presence of an extra, unseen, channel (i.e. assuming that the total number of active channels is really N+1), allows us to calculate the probability of there being no events recorded in which all channels were simultaneously open (no level N+1 events, given N+1 channels active). For the top trace, this conditional probability (assuming the presence of a second channel) was below 10^{-5}, while for the bottom trace it was 0.92, suggesting that it is likely that three or more channels were contributing to the openings observed.

Chapter 20

Web Text Box 1.b

An electrophysiological study of a CFTR mutant

ANSWER TO THE ELECTROPHYSIOLOGICAL QUESTION