A Single or Dual Channel in Nerve Membranes?

Dear Sir:

In his recent letter to you, Dr. Mullins suggests that the evidence on squid axon membrane conductances said to be compatible with the notion of two independent ionic conductances for Na and K "is not at present compelling." We certainly agree with this and furthermore would point out that we do not yet see any experiment which could help us choose definitively between a single channel (of the Mullins' 1959 type) or a pair of independent channels.[1]

Dr. Mullins makes an interesting proposal for a conclusive experimental demonstration of two channels in the membrane; this would be an observation in which both ionic conductances were simultaneously and fully "turned on," giving rise to a total membrane conductance of twice the normal maximum value for either ionic species. We agree that perhaps this would be *sufficient* to establish the two-channel notion as the preferred theory. Mullins declares that such data have not been found, nor do we know of any. However, it does *not* appear *necessary* to *require* that both channels be simultaneously open, although they "both *could, in principle,* be opened at the same time." It is quite possible that an observation may be made in which there is some increase (e.g., 10–20%) in the total membrane conductance. Before this is said to strongly lend weight to the two-channel notion, the single channel hypothesis should be carefully examined to see whether it allows such a situation. For example, in his original treatment, Mullins (1959) set up different dispersions about the Na and K pore sizes in order to match the available experimental data on conductance. It is, in principle, possible that an agent could reduce the dispersion about the potassium pore size and produce twice the normal potassium conductance. Then the question should be raised as to whether or not this narrower distribution would also have an associated minor peak[2] at a pore size compatible with Na flow.

Although there is no single definitive experimental observation in hand, several

[1] There is also a leakage component (Hodgkin and Huxley, 1952) in the membrane currents of squid axons. Myelinated nerve fibers might be said to have "multichanneled" membranes, because there are at least four components including the leakage (Frankenhaeuser and Huxley, 1964).

[2] See Fig. 8 of Mullins (1959) where the sharp peak for Na was associated with a minor peak at a Rb pore size.

pieces of evidence taken together make it at least more convenient to conceive of separate channels because fewer additional assumptions are required. Some of this evidence is summarized below:

1. *The Independent Effect of Prehyperpolarization on the Kinetics of the Channels* Cole and Moore (1960) found that a strong hyperpolarization preceding a test pulse very significantly delayed the potassium turn-on but the early transient channel kinetics were not affected. This does not seem to fit into the original Mullins single channel model where the g_{Na}-off process is directly related to the g_K turn-on.

2. *The Selective Blockage of the Early Transient Conductance by Tetrodotoxin (TTX)* We (Narahashi, Moore, and Scott, 1964; Takata, Moore, Kao, and Fuhrman, 1966) have found that the early transient conductance change is reduced in amplitude without a change in kinetics and without any modification of amplitude or kinetics of the late (potassium) conductance change. Furthermore, we (Moore, Blaustein, Anderson, and Narahashi, 1967) have observed that TTX blocks the flow of any ions in either direction, through the "early transient channel" but does not affect the flow of any ions using the "late steady channel." TTX must not affect the channel kinetics of any model assumed but must act to change either the number of completely open channels or the average opening of all channels. Mullins suggests in his letter that whether any ions flow through his model of a single channel could depend on the modulating influence of substances present at the channel entrance on either side of the membrane. Although the action of TTX might be described by means of this additional assumption of flow modulation at the time when the pore is of the proper size to pass Na ions, one must further assume that TTX does not modulate the flow when the channel is at the potassium pore size.

3. *The Differential Changes in the Time Course of the Sodium-off Process and the Potassium Conductance Increase upon Treatment by DDT* For the present purposes, the observation (Narahashi and Haas, 1968) of a 4.5-fold slowing of the sodium current turn-off kinetics accompanied by only a slight slowing (1.4–1.6-fold) of the potassium onset is probably more important than the sum of the conductance maxima. Furthermore, Hille (1968) observed that some (about one-fourth of the sodium channels in frog nodes remain open indefinitely after depolarization in DDT, with no effect on rate or magnitude of the potassium channel opening. In the single channel model the sodium-off process is coupled with the potassium-on process. Again, it seems that additional assumptions would be necessary to account for the DDT observations.

In response to some minor points made in Mullins' letter, we have found that the effect of TTX is slowly but completely reversible upon washing with normal media (Narahashi, Haas, and Therrien, 1967; Narahashi, Moore, and Poston, 1967). As for the site of action of TTX, it has been suggested (Kao and Nishiyama, 1965; Narahashi, Anderson, and Moore, 1967; Moore et al., 1967) that the TTX molecule plugs the channel at the gate located on the external surface of the nerve membrane by virtue of its guanidinium group.

For some time we have sought definitive experiments to distinguish between the

single and dual channel concepts. We appreciate the comments by Dr. Mullins and welcome further discussion along this line.

Received for publication 8 May 1968.

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