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Pore dilation reconsidered

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Abstract

Previous experiments have suggested that many P2X family channels undergo a time-dependent process of pore dilation when activated by ATP. Li et al. now propose a different interpretation of the key experiments.

For most ion channels, the size of the pore appears to be fixed once the channel has opened. Some exceptions to this are clearly documented, most notably in the form of rare and transient ‘subconductance’ states¹. In general, however, such states either have the same ionic selectivity as the main open state or are occupied so briefly that they are unlikely to have any physiological significance. In this context, the apparent behavior of a group of ligand-activated cation channels is remarkable. In these channels—including P2X2 (refs. 2,3), P2X4 (refs. 2,3), P2X7 (ref. 4), TRPV1 (ref. 5) and TRPA1 (ref. 6)—the pore of the channel seems to undergo a striking change increase in permeability to large molecules in a time-dependent way. This has been referred to as “pore dilation.” As with almost all dynamic behavior of channels, the evidence for pore dilation has been based on inferences from electrical recordings of currents through the channels. In this case, the primary observation suggesting pore dilation is an apparent time-dependent change in the permeability ratio of large cations, such as *N*-methyl-D-glucamine (NMDG) or Tris, relative to small cations such as sodium or potassium. In whole-cell patch clamp recordings with an internal solution containing mainly sodium cations and an external solution containing NMDG, the reversal potential when channels are first activated by ligands is initially very negative, suggesting low permeability of NMDG⁺ relative to Na⁺, but shifts to progressively more depolarized values over seconds, suggesting an increase in NMDG⁺ permeability. If recorded at a constant voltage in between the two reversal potentials, the current is first outward (carried by Na⁺) and then inward (carried by NMDG⁺).

Li *et al.*⁷ now propose a completely different explanation for this behavior: that the time-dependent change in reversal potential, while very real, is not caused by a time-dependent change in channel permeability but rather by a dramatic change in the ion concentrations inside the cell—so that, for example, intracellular Na⁺ falls from 140 mM to 20 mM and intracellular NMDG⁺ increases from 0 mM to 200 mM. These changes are especially striking given that the intracellular solution is in constant contact with an essentially infinite reservoir of solution with the original composition, exchanged through the open pipette tip of the patch clamp pipette in whole-cell mode. The reason they occur, according to a detailed model that Li *et al.*⁷ present to support their interpretation, is that with high enough expression of channels in the membrane, cumulative exit of sodium ions through all the

channels in the cell is much faster than the ions can be replenished from the pipette, and similarly entry of NMDG⁺ through channels occurs faster than NMDG⁺ can diffuse into the pipette.

In addition to supporting their new interpretation by modeling, Li *et al.*⁷ present a number of experiments most easily explained by their interpretation. For example, they show that no change in reversal potential is seen if the channels are activated for many seconds with symmetric Na⁺ concentrations but then tested with the NMDG⁺_{out}/Na⁺_{in} condition. And perhaps most convincingly, they show that the change in reversal potential occurring with NMDG⁺_{out}/Na⁺_{in} can be reversed if external NMDG⁺ is replaced temporarily by external Na⁺. They also support their modeling by making measurements of the depletion of intracellular K⁺ using coexpressed potassium-selective channels.

The phenomenon of time-dependent changes in reversal potential resulting from unexpected changes in concentration of permeant ions has a long history. Frankenhaeuser and Hodgkin⁸ proposed that a time-dependent depolarizing shift in the reversal potential of the delayed-rectifier conductance of the squid giant axon, and a similar depolarizing shift in the after hyperpolarization of repetitive action potentials, could be explained by accumulation of potassium ions in a restricted space between the axon and its Schwann cell sheath and provided a detailed model accounting quantitatively for the phenomenon. However, while it is easy to see how a concentration of ions that starts off low can increase substantially by accumulation in a restricted space, it is much less intuitive that ions with an initial concentration of 140 mM in the relatively large volume of a cell can be depleted substantially by flow through membrane channels, especially in a cell whose contents are in contact with a huge reservoir of the same solution in a patch pipette. Yet the data and model of Li *et al.*⁷ convincingly argue that this can occur. In fact, evidence that apparent changes in reversal potential can result from ion depletion in internal solutions during whole-cell recordings was described 15 years ago by Frazier *et al.*⁹ in studies of voltage-dependent potassium channels, although in that case the absolute concentration changes involved were much smaller.

It is important to emphasize that the re-interpretation offered by Li *et al.*⁷ does not question that P2X, TRPV1 and other channels are unusual in having large pores that can permeate very large cationic molecules. Rather, the re-interpretation is that the permeability to large cations is present immediately on activation of the channel, and with the same time course as permeability to small cations. This is consistent with other measurements showing immediate activation of currents carried by large cations in TRPV1 channels¹⁰. A wealth of evidence showing entry of high-molecular-weight fluorescent dyes through P2X (refs. 2–4,11,12) and TRPV1 (ref. 5) channels is also not in question; sometimes there is an apparent delay in entry, but it is hard to know whether this reflects delayed entry or a threshold for detection.

The experiments and model provided by Li *et al.*⁷ make a convincing case that remarkably large-scale changes in intracellular ion concentrations can occur as a result of membrane current flowing during whole-cell recording and can produce changes in reversal potential of the type that have been interpreted as pore dilation. Of course, this does not prove that

genuine pore dilation cannot occur. Li *et al.*⁷ analyzed only P2X channels and only under particular experimental conditions. Considering the long history of experiments interpreted in terms of pore dilation, now dating back 16 years, and the detail and complexity of some of the protocols and models used to support it¹², it may be unlikely that all interested scientists will be immediately convinced that the concept is dead, and many workers are likely to carefully scrutinize the conditions, protocols and model of Li *et al.*⁷. One can imagine experiments that could show pore dilation more directly, including time-dependent changes in single-channel currents, especially those carried by large cations, or finding single-channel conductances that depend on ligand concentration. Showing time-dependent changes in reversal potential under conditions where changes in ion concentrations should be minimal, such as with low expression levels of channels or perhaps with planar membranes with compartment geometries less susceptible to ion depletion, could also be convincing. Refining our electrophysiological understanding of ion permeation of P2X and TRP channels is particularly timely because the recent acceleration of progress in obtaining high-resolution structures by cryo-electron microscopy has already started to provide structures of these and related channels in open states¹³⁻¹⁴, opening an entirely new avenue for understanding permeation in these fascinating channels.

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