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New and Notable

Gap Junction Channels: Yes, There Are Substates, But What Does That Mean?

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One of the frustrations of working with gap junction channels is the necessity to view single channel properties by voltage clamping cell pairs. Not only does the burden of input resistance and capacitance make our recordings of junctional channel activity noisier and less crisp than those of nonjunctional channels ripped from the surface on small patches of membrane, but it also makes it very difficult to perform serious steady-state analysis of channel open and closed times, because most gap junctions have just too many channels with high open probabilities in their plaques. Thus, most of what we know of the sizes of junctional channels has come from studies where overall conductance has been reduced reversibly by pharmacological interventions. Such studies on mammalian systems have revealed characteristic conductance values for gap junction channels made of different connexin proteins, although multiple channel sizes are present in most studies—even on transfectants where a single connexin is expressed. It has thus remained ambiguous whether the multiple channel sizes might either reflect the presence of multiple connexins or of single types of gap junction channels but with subconductance states. To resolve this issue and to gain mechanistic insight into junctional gating, experimental preparations with a small number of operational gap junction channels are clearly desirable. Unfortunately, naturally occurring preparations with only a few operational channels are rare (Chanson et al., 1993).

Experimental strategies to circumvent this problem have included attempts to patch junctional membranes directly (Manivannan et al., 1992), but that technique has so far been hampered by the strict requirement that the channels recorded must be proven to be truly junctional. And at any rate, junctional patching appears to be limited to cells (such as *Xenopus* oocytes) large enough that the region of former junctional contact is recognizable after one cell is ruptured. Another technique that limits the number of functional channels is that employed by Bukauskas and Weingart (1994), who have resurrected the use of reaggregating dissociated cells.

Reuniting isolated cells has a rich history in the gap junction field, having been used to study structural changes in junctional particles and appositional membranes (so-called "formation plaques") during gap junction formation, to provide information on lateral diffusion of channel precursors ("hemichannels" or "connexons"), and to demonstrate that only a small number of functional channels is required to synchronize spontaneous activity in excitable cells. Reaggregation has also been used previously by Drs. Weingart and Bukauskas and others to reveal single channel currents in gap junctions of both arthropods and vertebrates. The advantage of such a preparation for the study of single channels is that channel formation occurs sigmoidally with time; before the rate of channel accrual reaches its maximum (in this system, about 3 channels/min), there is a window of opportunity when only a single channel exists, then another is added, and so on. Although the period of time is limited in which a very small number of channels are present (and may be inadequate for detailed characterization of channel kinetic properties), the high input resistance of the insect cell line and the large unitary conductances of the junctional channels investigated in this study allow exquisite resolution of the individual transitions, revealing unexpected behavior of the channels as the cells begin to communicate.

The result is a finding of general interest to gap junctionologist and channelologist alike: a gap junction channel forms its intercellular connection before it opens, and after the channel is sealed off from extracellular space it transits through a low-conductance state before attaining the main open-channel conductance. This channel substate is also preferentially occupied when high transjunctional voltages (V_js) are imposed, and intermediate unitary conductance values are detected at intermediate V_js. Also interesting is the finding that other factors (in the case of most arthropod channels, closure occurs upon cell depolarization, regardless of V_j) can gate the main state without obligate occupancy of the subconductance state, reinforcing the old concept that different gating mechanisms may operate through distinct conformational changes. That this finding is generalizable to the vertebrate connexins was shown in last month's issue of *Biophysical Journal*, where the cardiac gap junction connexin was shown to possess a substate at high V_j that was not entered into upon closure by halothane (Moreno et al., 1994); it is apparently this substate that is responsible for the prominent voltage-insensitive component of junctional conductance (termed "g_{min}") that has now been observed for gating by V_j in numerous cell types.

Now that gap junction substates are out in the open, a question that is begging an answer is whether the reduced conductance is associated with restricted permeability or with altered charge selectivity. Although in excitable tissues gap junction channels primarily function to exchange small cations (termed "ionic coupling"), their role in most cells is presumably the exchange of second-messenger molecules (termed "metabolic coupling" or "metabolic cooperativity"), many of

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which are large anions (e.g., cAMP, IP_3). Therefore, if different connexins or different conductance states of the same channel are associated with different selectivities (see Veenstra et al., 1994), modulated isoform expression could become a major control mechanism operating to form compartmental boundaries within tissues.

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