Commentary

Single Channel Seeks Permeant Ion for Brief but Intimate Relationship

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Ion channels don't like having their permeant ions taken away. Particularly when they are in the mood to let ions through, this sort of deprivation makes them feel downright unstable. Like jilted lovers, they shut the door and become listless, inactive(-ated), and sometimes even immobilized. Channel physiologists have long tried to ignore this dejected behavior on the part of the channels, even though for many years it has been clear that channels often refuse to gate normally in unfriendly ionic conditions. The best known such effect is the slowed closing of channels in the presence of high concentrations of certain permeant ions, often called the "foot in the door" effect (Yeh and Armstrong, 1978). This type of effect was first noticed for synaptic channels (Ascher et al., 1978; Marchais and Marty, 1979) and has been studied in detail for a variety of K^+ channels (Stanfield et al., 1981; Swenson and Armstrong, 1981; Matteson and Swenson, 1986; Shapiro and DeCoursey, 1991; Neyton and Pelleschi, 1992; Demo and Yellen, 1992).

Three recent papers in this journal highlight the different possible manifestations of such ion effects on gating and emphasize their importance for a good biophysical understanding of the channel mechanisms. Cloned voltage-activated Na^+ channels show two distinct changes in gating when extracellular Na^+ ions are replaced by impermeant ions (Townsend et al., 1997; Townsend and Horn, 1997). The first change is that their open probability is reduced, particularly for very large depolarizations. This decrease is not seen with normal concentrations of extracellular Na^+ and is likely to result from a voltage-dependent depletion of ions from the pore. Only the highly permeant ions Na^+ and Li^+ can prevent this effect, which does not depend on the normal fast inactivation mechanism. In fact, it is more prominent in a mutant with reduced fast inactivation. Townsend et al. (1997) show that the ion-sensitive steps are very rapid, and they argue that the most likely explanation of the effect is that, in the absence of extracellular permeant ions, there is a rapid inactivation that competes with the opening process.

The second effect of reduced Na^+ concentration is to enhance Na^+ channel slow inactivation (Townsend and Horn, 1997). In low $[Na^+]$, the onset of inactivation is faster and recovery is slower. In contrast with the rapid ion effects on open probability, all of the alkali cations tested can oppose slow inactivation, even the weakly permeant K^+ and Cs^+ ions.

Both of these effects on Na^+ channels have analogs in the history of ion effects on K^+ channels. For a number of K^+ channels, reducing external $[K^+]$ can markedly reduce the open probability (Pardo et al., 1992; López-Barneo et al., 1993). It is not clear that these effects have the same rapid time course as the Na^+ channel effects, but they too are proposed to result from closed-channel inactivation. External $[K^+]$ can also modulate both onset and recovery from slow (C-type) inactivation (López-Barneo et al., 1993; Levy and Deutsch, 1996); the effects on the onset of inactivation are particularly marked under conditions where K^+ efflux through the channel is reduced by N-type inactivation or blockade (Baukrowitz and Yellen, 1995, 1996).

Townsend et al. (1997) predict that the ion effects on open probability may have significant effects on the gating currents, since inactivated states often produce a dramatic slowing or "immobilization" of gating charge movement. Their prediction appears to have been borne out in the third recent paper on Shaker-family K^+ channels (Chen et al., 1997). The problem with studying ion effects on gating charge movement is that to measure gating currents it usually is necessary to eliminate ionic currents. This is generally done either by removing permeant ions, adding blockers (which often act at the same sites as permeant ions), or using "nonconducting" mutants (which by definition disrupt some of the normal interactions of permeant ions with the channel). Chen et al. (1997) overcome this problem, partially, by measuring gating currents at or near the reversal potential for ionic currents in the presence of low concentrations of K^+ or the weakly permeant ion Cs^+.

To understand their results, it is necessary first to know what the previous gating current measurements have shown, in the absence of permeant ion interactions. Depolarizing voltage steps produce outward gating current ("on" currents) and the return voltage step produces a restoring "off" gating current; the net charge (integral of current $\times$ dt) for on and off charge movement is generally equal and opposite. For small or brief depolarizations (too small to produce much channel opening), the rate of off charge movement is quite rapid.

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However, for larger and longer depolarizations that would open the channels, the off charge movement becomes much slower. This has previously been interpreted to mean that the rate-determining opening step involves a concerted motion of the channel subunits to a particularly stable open state, which slows down the return of the off charge (Bezanilla et al., 1994; Zagotta et al., 1994).

Chen et al. (1997) find that in the presence of permeant ions this slowing of the off gating current is not seen, even though it is clear that the channels indeed are opening. This could be explained by supposing that permeant ions just speed up the closing of the open state, but it seems more natural to explain it by revising the original interpretation of the slow off currents. Chen et al. (1997) propose that the original observation of slowing actually is due to a rapid inactivation process, which predominates when permeant ions are absent (much like the rapid inactivation inferred by Townsend et al., 1997) in the absence of extracellular Na^+. K^+ ions cannot prevent the development of slow off currents in the nonconducting W472F mutant of Kv1.5 channels, which may be explained simply by the inability of ions to interact with the nonconducting channel. If these interpretations are correct, they argue for considerable caution in combining information obtained under widely varying experimental conditions (e.g., gating current measurements and ionic current measurements) to construct coherent models of channel gating.

Is there any physiological importance to these permeant ion effects on the gating of voltage-dependent channels? With rare exceptions, such as the taste buds, the concentrations of permeant ions around voltage-dependent channels in animal cells are carefully regulated by homeostatic mechanisms. This precludes most of the ion effects that are provoked by the dramatic manipulations of channel biophysicists; e.g., when they measure gating currents. However, this regulation can fail to some extent, particularly in the case of extracellular $[K^+]$: local accumulation during neuronal activity can produce increases of $[K^+]$ up to 6–10 mM (Sykova, 1983), and the changes might be even more pronounced in ischemic conditions. These $[K^+]$ changes may be enough to produce changes in open probability of certain K^+ channels (Pardo et al., 1992), particularly in the rate and extent of C-type inactivation (López-Barneo et al., 1993; Baukrowitz and Yellen, 1995, 1996).

What can we learn from these effects about ion channel structure and mechanisms? The theme that pervades all three of the effects described here and almost all the ion effects in the literature is that permeant ions stabilize the open channel structure. Why is it so common for open pore stability to depend on permeant ions? Perhaps the most obvious explanation is that these channels evolved to work under particular ionic conditions; because charged permeant ions have substantial energetic interactions with the open channel structure, these were included in the structural and energetic "design" of the channel proteins in the open state. But why aren't the open channel structures "overstabilized"? Why don't they have a design safety factor that allows pore stability to be maintained even under stress? One can propose two teleological answers to this question. First, the inherent instability or metastability of the open state produces a variety of inactivation mechanisms, which apparently prove to be useful at times. Second, if some of the interaction energy between the permeant ion and the channel is used to stabilize the open channel structure (à la Jencks, 1975), the net binding energy of the ion will be weaker. Perhaps, like multi-ion occupancy, this allows the channel protein to have strong and selective interactions with its favorite ion without binding the ion so strongly as to prevent rapid permeation.

REFERENCES

Ascher, P., A. Marty, and T.O. Neild. 1978. Lifetime and elementary conductance of the channels mediating the excitatory effects of acetylcholine in *Aplysia* neurones. *J. Physiol. (Camb.).* 278:177–206.

Baukrowitz, T., and G. Yellen. 1995. Modulation of K^+ current by frequency and external $[K^+]$: a tale of two inactivation mechanisms. *Neuron.* 15:951–960.

Baukrowitz, T., and G. Yellen. 1996. Use-dependent blockers and exit rate of the last ion from the multi-ion pore of a K^+ channel. *Science (Wash. DC).* 271:653–656.

Bezanilla, F., E. Perozo, and E. Stefani. 1994. Gating of *Shaker* K^+ channels: II. The components of gating currents and a model of channel activation. *Biophys. J.* 66:1011–1021.

Chen, F.S.P., D. Steele, and D. Fedida. 1997. Allosteric effects of permeating cations on gating currents during K^+ channel deactivation. *J. Gen. Physiol.* 110:87–100.

Demo, S.D., and G. Yellen. 1992. Ion effects on gating of the Ca^{2+}-activated K^+ channel correlate with occupancy of the pore. *Biophys. J.* 61:639–648.

Jencks, W.P. 1975. Binding energy, specificity, and enzymic catalysis: the Circe effect. *Adv. Enzymol. Relat. Areas Mol. Biol.* 43:219–410.

Levy, D.I., and C. Deutsch. 1996. A voltage-dependent role for K^+ in recovery from C-type inactivation. *Biophys. J.* 71:3157–3166.

López-Barneo, J., T. Hoshi, S.H. Heinemann, and R.W. Aldrich. 1993. Effects of external cations and mutations in the pore region on C-type inactivation of Shaker potassium channels. *Receptors Channels.* 1:61–71.

Marchais, D., and A. Marty. 1979. Interaction of permeant ions with channels activated by acetylcholine in *Aplysia* neurones. *J. Physiol. (Camb.).* 297:6–45.

Matteson, D.R., and R.P. Swenson. 1986. External monovalent cations that impede the closing of K^+ channels. *J. Gen. Physiol.* 87: 795–816.

Neyton, J., and M. Pelleschi. 1991. Multi-ion occupancy alters gating in high-conductance, Ca^{2+}-activated K^+ channels. *J. Gen. Physiol.* 97:641–665.

Pardo, L.A., S.H. Heinemann, H. Terlau, U. Ludewig, C. Lorra, O. Pongs, and W. Stühmer. 1992. Extracellular K^+ specifically mod-

ulates a rat brain K^+ channel. *Proc. Natl. Acad. Sci. USA*. 89:2466–2470.

Shapiro, M.S., and T.E. DeCoursey. 1991. Permeant ion effects on the gating kinetics of the type *L* potassium channel in mouse lymphocytes. *J. Gen. Physiol.* 97:1251–1278.

Stanfield, P.R., F.M. Ashcroft, and T.D. Plant. 1981. Gating of a muscle K^+ channel and its dependence on the permeating ion species. *Nature (Lond.).* 289:509–511.

Swenson, R.P., and C.M. Armstrong. 1981. K^+ channels close more slowly in the presence of external K+ and Rb+. *Nature (Lond.).* 291:427–429.

Sykova, E. 1983. Extracellular K^+ accumulation in the central nervous system. *Prog. Biophys. Mol. Biol.* 42:135–189.

Townsend, C., H. Hartmann, and R. Horn. 1997. Anomalous effect of permeant ion concentration on peak open probability of cardiac Na^+ channels. *J. Gen. Physiol.* 110:11–21.

Townsend, C., and R. Horn. 1997. Effect of alkali metal cations on slow inactivation of cardiac Na^+ channels. *J. Gen. Physiol.* 110:23–33.

Yeh, J.Z., and C.M. Armstrong. 1978. Immobilisation of gating charge by a substance that simulates inactivation. *Nature (Lond.).* 273:387–389.

Zagotta, W.N., T. Hoshi, and R.W. Aldrich. 1994. *Shaker* potassium channel gating. III: Evaluation of kinetic models for activation. *J. Gen. Physiol.* 103:321–362.