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Opening Ion Channels: Enzyme at the Gate

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Electrical signals propagate rapidly along the membrane of living cells and control various processes including muscle contraction, cardiac rhythm, hormone release, sensation, and information processing. At the molecular level, these electrical signals are mediated by voltage-gated ion channels, a superfamily of pore-forming membrane proteins that are ubiquitously expressed in excitable and nonexcitable cells across a wide range of organisms (for example, animals, plants, yeast, and bacteria). Voltage-gated ion channels respond to changes in membrane potential and allow ions such as Ca^{2+}, Na^{+}, and K^{+} to traverse rapidly across the membrane according to concentration and electrochemical gradients. Voltage-gated K^{+} channels (K_v) are formed by tetramerization of four identical or homologous subunits, each containing six transmembrane segments (S1–S6) with intracellular N- and C-termini. On the other hand, voltage-gated Ca^{2+} or Na^{+} channels contain four homologous domains, with each domain equivalent to a single subunit of the K_v channels. The ion conduction pore is formed by the fifth and sixth trasmembrane segments (S5 and S6), and voltage sensing is conferred largely by the fourth transmembrane segment (S4). In the S4 segment, five to seven positively charged arginine or lysine residues relocate within the membrane electric field upon changes in membrane potential. This rearrangement leads to conformational changes of the pore and renders channel opening or closing.

Historically, an extremely valuable tool for studying ion channel function has been natural venoms—agents employed for self-defense or aggression by toxic species of scorpions, fish, snakes, bees, spiders, and cone snails. For example, tetrodotoxin from the puffer fish was used to first purify Na^{+} channels.[1] Currently, toxins are widely used to specifically label ion-channel proteins to study tissue distribution, to enable pharmacological differentiation of ion channel types, and as molecular calipers to probe structure and function of ion channels. Quite generally, toxins are believed to alter ion channel function by either blocking the ion conduction pore or modifying channel gating. However, a recent study by Ramu and colleagues at the University of Pennsylvania present a novel mechanism by which spider venoms regulates voltage gated K^{+} channels.[2]

Venoms from spiders of the *Loxosceles* species (brown spiders) can produce severe clinical symptoms including dermonecrosis, thrombosis, haemolysis, vascular leakage, and in severe cases, renal failure and death especially in children.[3,4] Although the venom contains several proteins, the phospholipase sphingomyelinase D (SMaseD) is of particular interest as it is responsible for dermonecrosis, hemolysis, blood vessel damage, and fibrogenolysis caused upon evenomation by *Loxosceles* spiders. SMaseD plays a key role in the metabolism of lipids. First, it catalyzes the conversion of sphingomyelin in the outer leaflet of the plasma membrane to the negatively charged ceramide-1-phosphate by removal of the positively charged choline group. Second, SmaseD hydrolyzes albumin-bound lysophosphatidylcholine (LPC) by removal of the choline head group to yield lysophosphatidic acid (LPA). As LPA is a potent lipid mediator known to mediate a variety of physiological and pathological responses by signaling through G protein-coupled receptors and activating many intracellular signaling pathways, the pathology associated with brown recluse spider envenomation has been attributed to LPA production. In the study by Ramu et al., it was suggested that the sphingomyelin reaction catalyzed by SMaseD contributes to the pathological responses possibly by activation of K^{+} channels. By testing various fractions of venom from the brown recluse spider, the authors found that SMaseD induced robust outward current through a voltage gated K^{+} channel ($K_v1.2$) at resting membrane potential, where these channels would otherwise be nonconductive. In experiments to further confirm the interaction, recombinant SMaseD was prepared and demonstrated to activate $K_v1.2$ channels. Moreover, It was shown that the $K_v1.2$ activation depended on Mg^{2+}, a cation critical to the enzymatic activity of SMaseD. The question thus arises as to how an enzyme activates a voltage gated ion channel.

Opening of voltage gated K_v channels requires membrane depolarization. From analysis of channel kinetics, it was shown that SMaseD shifted the threshold required for channel opening to near resting membrane potentials, at which channels are normally closed. The authors hypothesized that this effect was due to the metabolism of spingomyelin catalyzed by SMaseD. Spingomyelin is present mainly in the outer leaf of the lipid bilayer. Cleaving choline from sphingomyelin yeilds the negatively charged lipid ceramide-1-phosphate in the outer surface (Figure 1), which is equivalent to a membrane depolarization signal. In other words, SMaseD changes the lipid and membrane potential field surrounding channel protein leading to channel opening. Consistent with this idea, is the neutralization of the negative charges of ceramide 1-phosphate by adding extracellular cations

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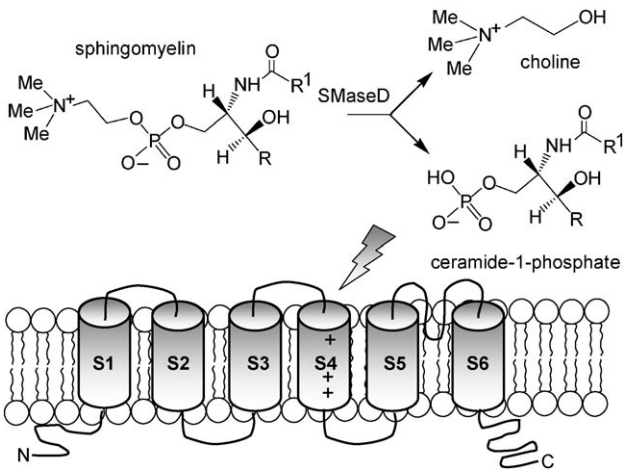

Figure 1. Schematic representation of a K^+ channel and hydrolysis of sphingomyelin catalyzed by SmaseD. R and R^1 indicate acyl chains. SMaseD catalyzes a reaction where the positively charged choline group of sphingomyelin is removed yielding ceramide 1-phosphate.

(Mg^{2+}) was able to negate the effect of SMaseD. Intriguingly, the effect of SMaseD varied between different Kv channels. Whereas SMaseD activated Kv2.1 at resting membrane potentials, smaller effects were observed on *Shaker* K^+ channels (only about −10 mV shift in current-voltage relationship and gating charge-voltage relationship). Furthermore, no noticeable effect on mEAG or mSlo K^+ channel was observed. These observations are somewhat surprising, as a simplistic assumption would be that SMaseD results in similar changes of the lipid environment thereby affecting ion channel properties. A more plausible explanation is that ceramide-1-phosphate may interact specifically with specific residues on the ion channel protein. It will be of interest to determine if and how charged residues interact with ceramide-1-phosphate, and to what extent these interactions affect ion channel gating. Also of interest is whether SMaseD can affect the function of other ion channels, particularly voltage-gated calcium channels and sodium channels.

The results of this study reveal a novel mechanism by which SMaseD opens voltage gated K_v channels by altering their lipid environment. This previously unknown mode of activation of voltage-gated ion channels provides additional insight into how cellular excitability may be regulated physiologicaly or pathologically by altering properties of the lipids on the plasma membrane. It also sheds light on how voltage-gated ion channels might be activated in nonexcitable cells. These observations point to the importance of lipid–ion channel interactions and open avenues to delineate specific interactions. Perhaps even more important, this finding brings forth additional avenues, hitherto unexploited, for enabling ion channel drug discovery. The heightened interest in pursuing voltage gated ion channels as drug targets, together with the increased emphasis on identifying compounds with exquisite subtype selectivity, has generated considerable efforts in the development of robust ion channel screening assays. Functional assays such as ion influx, membrane potential, and whole cell patch clamp are typically utilized for screening and lead optimization of ion channel active compounds. However, development of functional assays for voltage gated channels remains challenging, especially in a high throughput mode. This is due to the fact that voltage gated ion channels do not typically have chemical activators, and manipulation of membrane electrical field in a high throughput manner remains technically challenging. The ability of SMaseD to activate voltage gated ion channels by altering their lipid environment may offer an opportunity to overcome this hurdle and create a new paradigm for screening voltage-gated ion channel targets.

Keywords: gating · ion channels · *loxosceles reclusa* · potassium · sphingomyelinase D

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