Heatlines The Science of Sensation

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### Bio-electricity and the sensing of electric fields

# Feel the force

In an issue devoted to sensory phenomena, it may seem odd to include an article on sensing something that we cannot consciously perceive: electric force. Of course, we can sense the dramatic power of a lightning bolt: we see the flash, hear the boom, feel the rumble, and, if we're close enough, smell and taste the ozone produced. Lightning is caused by an enormous electric field that develops under a thundercloud due to the separation of electrical charges between the cloud and the earth. Almost 250 years after Benjamin Franklin's kite-in-the-storm experiment, we still don't fully understand how this charge separation is generated. But it's nevertheless a fact that if you are standing under a thundercloud, you are immersed in a large vertically directed electric field, typically a few hundred volts between your head and your feet. Just before a lightning strike, this electric field becomes large enough to literally rip gas molecules in the air apart by pulling negatively charged electrons in one direction - towards the positively charged ground - and positive nuclei towards the negative cloud. Once a column of air becomes ionized in this way, the charged particles zoom to their respective 'electrodes', discharging the cloud-ground capacitor in a bright, hot flash of enormous electrical current. Lightning is a manifestation of a phenomenon called 'dielectric breakdown', something we've all seen in more controlled contexts, such as cheesy horror movies, Ask-Dr-Science demos at science museums and aluminium foil mistakenly placed in the microwave oven. Dielectric breakdown of most materials occurs at electric fields on the order of a few million volts per metre. But there is a great irony here: whereas we sense all of the secondary consequences of lightning, we are utterly blind to its most fundamental element – the electric field that gets the whole thing going. Humans just never evolved electric-force sensors. (But fish did – and migratory birds and turtles navigate by 'feeling' the earth's weak magnetic field.)

#### **Electricity in cell membranes**

So what is there to discuss? It turns out that, although we humans cannot feel electric forces, our cells most certainly do. The thin lipid membranes surrounding every cell are, electrically speaking, like the air-gap between thundercloud and ground. Cell membranes contain energy-consuming electricity-generating power plants: ion pumps, electron-transport enzymes, ion channels and other proteins that separate charged particles - Na<sup>+</sup>, K<sup>+</sup>, H<sup>+</sup>, Ca<sup>2+</sup> and Cl<sup>-</sup> - across the membrane. As a result, biological membranes sustain voltages typically of the order of 50 mV, cytoplasmic side negative<sup>1</sup>. That doesn't sound like much - less than a twentieth of a torch battery. But it's not the voltage itself that is felt by electric charges inside the membrane, but rather the electrical force - the spatial gradient of voltage - volts per distance, otherwise known as the electric field. A simple calculation tells us that electric fields within cell membranes are really really big: 50 mV falling over a membrane's ~40× width is over a million volts

per metre. That is within an order of magnitude of dielectric breakdown, not far off the threshold for triggering a lightning bolt.

This means that integral membrane proteins must deal with something that soluble proteins never have to worry about – tremendous electrical forces. Every arginine side chain, for instance, that finds itself in a transmembrane region feels a strong pull towards the cytoplasmic side of the membrane, and every glutamate towards the cell's exterior; the membrane-embedded protein, if not careful about where its charged residues are located, would be in danger of being twisted into a molecular pretzel.

#### An example from human physiology

Although all membrane proteins have to survive in this strong electrostatic environment, some have evolved to exploit it – to 'sense' transmembrane voltage and respond to it. We'll consider a common bit of human physiology to illustrate the point (Figure 1). You have just gobbled up a super-sized piece of chocolate cake,

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## Features



**Figure 1.** Signalling from blood glucose to insulin release. After eating, glucose is used to produce ATP via glycolysis inside  $\beta$ -cells. The spike in ATP blocks the K-ATP channel, stopping potassium flow out of the  $\beta$ -cells. This in turn causes a build up of positive charge in the cell which leads to cell depolarization. The change in membrane potential causes voltage-sensitive Ca<sup>2+</sup> channels to open, and the influx of Ca<sup>2+</sup> leads to insulin secretion.

and, as a result, your blood glucose increases from its pre-meal level of ~4 mM to a spike of ~10 mM. Your body's cells need to be stimulated to sop up and metabolize this fuel, and the signal to do that is a big increase in the level of circulating insulin that occurs after a meal. But how does your pancreas, where insulin is stored, know that it's time to release this hormone into the bloodstream? That's where the electrical action of cells, and voltage-sensing in particular, is crucial<sup>2</sup>. The membranes of insulin-releasing pancreatic  $\beta$ -cells contain a pair of membrane proteins that link blood glucose levels to insulin release: an ATP-sensitive K<sup>+</sup> (K-ATP) channel and a voltagesensing Ca2+ channel. The glucose spike revs up glycolysis in the  $\beta$ -cell, whose intracellular ATP rises as a result. This increased ATP binds to and turns off the K-ATP channel. As a result, the outward flow of K<sup>+</sup> that normally sustains the cell's cytoplasm-negative voltage is diminished, and the voltage becomes less negative. A voltage-sensitive Ca2+ channel now 'feels' this change in transmembrane electric field, and 'responds' by opening a Ca<sup>2+</sup>-specific pore through the protein. Ca2+ ions, which are at a much higher concentration outside the cell than inside, spill into the cell through this pore, and the resulting increase in cytoplasmic  $Ca^{2+}$  signals the  $\beta$ -cell to dump its insulin, sequestered in cytoplasmic packets, into the blood.

We'll ignore the many complexities of this elaborate physiological process. Instead, we focus upon a key feature of insulin release: the Ca2+ channel's ability to 'know' when it's time to open and thus provide the Ca<sup>2+</sup> that triggers insulin release. This protein is constructed from two distinct membraneembedded domains (Figure 2): the voltage-sensing domain (VSD), formed from four transmembrane helices (S1-S4) in the N-terminal part of the polypeptide, and the pore-domain, formed from two transmembrane helices in the C-terminal part<sup>3</sup>. The channel is a complex of four of these units arranged around a central axis, like the staves of a barrel, with the VSD positioned on the periphery of the central pore-domain. One of the helices in the pore-domain acts as a gate that can either swing inwards to block off the cytoplasmic end of the pore or splay outwards or open it. Thus Ca2+ channels can adopt either of two basic conformational states: 'open', which allows Ca2+ permeation, and 'closed,' which is nonconducting. In the pancreatic  $\beta$ -cell, Ca<sup>2+</sup> channels are normally closed at the negative resting voltage that prevails before lunch, when your blood glucose is low, but they are stimulated to open when the voltage becomes less negative, or, in electrical jargon, when the cell 'depolarizes' in response to the increase in ATP following the chocolate cake.



**Figure 2.** Molecular architecture of Ca<sup>2+</sup> channel. Ca<sup>2+</sup> channel membrane-embedded domains are made up of the VSD, made up of four transmembrane helices, and the pore domain, made up of two transmembrane helices. Four of these subunits come together to form the functional channel with the VSD peripheral to the pore.

#### The voltage-sensing domain

We now come to the essential question: how does the Ca<sup>2+</sup> channel's pore-domain know that the membrane has depolarized? The answer is simple: the VSD feels the electrical forces associated with the voltage change and moves with them, tugging the pore-domain's gates open<sup>4</sup>. To visualize this process (Figure 3), we need to appreciate three essential features of the VSD. First, the VSD can adopt either of two stable positions with respect to the membrane: 'in', near the cytoplasmic side of the membrane, or 'out,' where it is closer to the extracellular side. Secondly, since the VSD is connected directly to the pore domain, its movement across the membrane is mechanically coupled to the opening/closing of the gate; the in-position of the VSD nudges the gate closed, and movement to the out-position drags it open. Thirdly, and this is the real kicker, despite its overall hydrophobic nature (as befits a membrane protein), the VSD carries three conserved arginine residues on each of the four units making up the channel - 12 positive charges in all. These charges feel real forces when placed in an electric field, and when the voltage changes, so will the forces on the VSD.

Let's see how these details determine the behaviour of  $Ca^{2+}$  channels in the  $\beta$ -cell. At rest, before the meal, remember that the cell is electrically polarized, negative inside. The electric field in the membrane applies a strong force on those 12 membrane-embedded arginine residues, a force that keeps the VSD in its in-position, which in turn holds the pore domain's gate closed. But when the sugar spike depolarizes the membrane, the electric field falls towards zero, and the loss of electrical pull to the inside liberates the VSD to visit its outposition. When the VSD moves outwards, it pulls the gate open, allowing Ca2+ to rush into the cell and stimulate insulin release. In this way, membrane depolarization caused by ATP-inhibition of K-ATP channels leads to flow of Ca2+ into the cell.

#### VSDs are found everywhere

The involvement of  $Ca^{2+}$  channels in insulin release is just one example of voltage-sensing membrane proteins.  $Ca^{2+}$  channels belong to a huge superfamily of ion channels, each type specific for different biological cations such as Na<sup>+</sup>, K<sup>+</sup>, H<sup>+</sup> and Ca<sup>2+</sup>, each carrying arginine-bearing VSDs built along the lines described above. These channels find themselves used for a multitude of biological purposes. The generation of electrical impulses in neurons by the co-ordinated action of Na<sup>+</sup> and K<sup>+</sup> channels is the context in which voltage-sensing in biology was

#### Voltage-dependent equilibrium in a membrane

The treatment below is oversimplified, but it exposes the essence of all voltage-dependent conformational changes of membrane proteins: the movement of charges in an electric field. Suppose that a membrane protein consists of two parts – a 'catalytic' domain (e.g. ion channel pore or phosphatase domain) and a VSD. Further suppose that this protein can exist in either of two conformations that describe the protein's functional character – 'inactive' (I) and 'active' (A). Finally, suppose that the VSD moves its position in concert with the conformational change, such that *z* positive charges move across the entire membrane from inside to outside upon going from I to A.

First, we consider the conformational equilibrium constant of the reaction  $I \rightarrow A$  when the *voltage across the membrane is zero*. This equilibrium constant,  $K_{o'}$ , is a direct indicator of the amount of activity under this zero-V condition, and it reflects the protein's intrinsic chemical preference for either conformation, as expressed in the standard Gibbs free energy of the transition:

$$K_0 = [A]_0 / [I]_0 = \exp(-\Delta G^{\circ} / RT)$$

A very negative (favourable) value of  $\Delta G^{\circ}$  means that the protein greatly prefers to be in A at V=0 (as is the case for the Ca<sup>2+</sup> channel discussed in the text).

Now consider the conformational change occurring when a voltage is applied across the membrane. In this case, the VSD will feel electrical forces pulling it inward (for negative voltage) or outward (for positive voltage). This effect is expressed thermodynamically by simply adding this 'extra' electrostatic energy to the intrinsic chemical Gibbs free energy of the transition:

$$\Delta G^{\rm total} = \Delta G^{\circ} - zFV$$

where F is a 'fudge factor' – the Faraday constant – that converts coulombs of charge into moles of charge, and the minus sign accounts for the fact that positive voltages make the outward movement of the VSD more favourable (more negative Gibbs free energy). Now, we can immediately write down the equilibrium constant of the conformational transition at any voltage:

#### $K(V) = K_0 \cdot \exp(zFV/RT) \sim K_0 \cdot \exp(zV/25 \text{ mV})$ at room temperature

Thus, as *V* is made increasingly negative, the protein's activity increases in an exponential fashion. The sensitivity of this change depends on how much charge is transferred when the VSD moves, i.e. the value of *z*. If, as with many ion channels,  $z \approx 10$ , this is a very large effect. For instance, if the Ca<sup>2+</sup> channels in the  $\beta$ -cell are open 99.9% of the time at V = 0 ( $K_0 = 1000$ ), then at V = -50 mV, they will be open only  $\sim 10^{-6}$  of the time. That is, a voltage excursion from -50 mV, the low-glucose condition of the  $\beta$ -cell, to near zero voltage, the high-glucose condition, brings the Ca<sup>2+</sup> channels from being nearly always closed to nearly always open.

originally discovered<sup>5</sup>. Mammalian photoreceptors reports the ambient the light level to the optic nerve via voltage-sensing  $Ca^{2+}$  channels, which more generally control exocytosis of neurotransmitters at synapses and of hormones in a variety of endocrine cells. Macrophages kill bacteria with proton channels that know by voltage changes when to pour out an acid-stream on to the unfortunate microorganism. The list goes on and on, throughout the biological world. Voltagesensing K<sup>+</sup> channels with completely unknown biological roles are found in archaea living in hydrothermal vents near 100°C.





**Figure 3.** Voltage-sensing by membrane proteins. The VSD is physically coupled to the pore and the membrane potential dictates where it sits in the membrane. Under depolarizing conditions, the VSD moves towards the outside of the membrane and pulls the pore opened. Under resting conditions, the VSD sits closer to the inside of the membrane, causing the pore to remain closed.

Of course, VSDs are found only in membrane proteins – where strong electric fields exist in biological systems. But it's not only in ion channels that VSDs are found. Recently, the first voltage-sensing enzyme, a membrane lipid phosphatase, was identified in a primitive marine chordate<sup>6</sup>. This protein has two domains – a classical VSD attached to a catalytic domain that hydrolyses phosphatidylinositol 4,5-bisphosphate with a rate controlled by – you guessed it – membrane voltage; we can only speculate at how VSD movement is coupled to enzyme activity in this case, but our deep knowledge of voltage-dependent ion channels provides plausible pictures, to be filled in when crystal structures come to light. At this point, we don't know whether other types of voltage-sensing membrane enzymes will be discovered – but I wouldn't bet against it.

In summary, voltage-sensing – or, more properly, electric field-sensing – is a key biological function without which biological electrical activity could not exist. It operates on a simple physical principle: that charge separation across a membrane leads to very large electrical forces on charged groups within the membrane. If a conformational change leading to functional activity of the

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protein – ionic flow through a channel or catalysis of an enzyme – is coupled to the movement of these charges, then – *voilà* – voltage-sensitivity automatically emerges – like lightning!



Chris Miller, a professor at Brandeis University and HHMI Investigator, enjoys studying the little details everyone else overlooks, and excels at showing why these little details matter. His work focuses on the

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