Ion Channels: Their Discovery and their Role in Pharmacology and Biomedicine

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In 1968, when I started my PhD project at the Max-Planck-Institute for Psychiatry in Munich, the mechanisms of nerve impulse generation were quite well understood on the macroscopic level. Hodgkin and Huxley (1952) in their groundbreaking work on the squid giant axon had shown that the membrane of this exceptionally large nerve fiber undergoes ion-specific permeability changes upon a depolarization, leading first to an influx of sodium ions, followed by an efflux of potassium ions. One of the pressing questions of that time was: What is the molecular mechanism of these permeability changes. Bert Sakmann, then a doctoral student in the Department of Otto Creutzfeldt, and myself, a student in the laboratory of Dieter Lux, often discussed the question how to prove the concept - already suggested by Hodgkin and Huxley - that the permeability changes come about through the opening and closing of small pores in the membrane of nerve cells. One possibility for such proof was to demonstrate that current changes discontinuously when such pores (or channels) open and close. Abrupt changes in current had already been demonstrated in artificial membranes, when these were doped with the pore-forming antibiotic gramicidin (Haydon and Hladky, 1972).

First Single Channel Recordings

We both went to different places for postdoctoral work, Bert Sakmann to London, joining Sir Bernhard Katz's laboratory, myself to Göttingen, to study gramicidin-induced currents in Hans Kuhn's Department at the Max-Planck-Institute for Biophysical Chemistry. It happened that Bert Sakmann, when returning from England, joined another department in the same institute. He meanwhile had obtained experience in the study of the neuromuscular junction of frog muscle. In particular, he had used digestive enzymes to remove the nerve terminal from the muscle surface and to expose the underlying postsynaptic membrane (Betz and Sakmann, 1973). This membrane was known to increase its permeability to sodium and potassium ions

when stimulated by the neuromuscular transmitter acetylcholine (Katz and Miledi, 1965). Also, evidence had been provided that currents flowing through single channels - if such entities existed - should be on order of magnitude of 1 pA (Anderson and Stevens, 1973). We soon realized that we might be able to reach our old goal of proving the channel concept by combining our expertise. We agreed that we should first tackle 'chemical excitability', since the exposed muscle membrane was supposed to contain a large number of acetylcholine-sensitive channels.

We started by using a method, which I had developed upon the suggestion of Dieter Lux, to measure local current density on the surface of nerve cells (Neher and Lux, 1969). In this method, a liquid-filled microscopic glass pipette is placed onto the surface with the goal of isolating and measuring the current, which flows through the 'patch' of membrane, covered by the pipette. Several laboratories used similar approaches at that time, with similar goals. One of the reasons, why we succeeded in the end, was the insight early-on, that the decisive parameter for achieving low-noise recordings was the quality of the contact between glass pipette and membrane. This requirement derives from the basic physical principle that any current flow displays fluctuations due to the discrete nature of ionic charges - electrons or ions. Thus, a current flowing through a resistor of resistance R is noisy with a mean noise amplitude δ of:

$$(1) \quad \delta = \sqrt{4 \bullet k_B \bullet T \Delta f / R}$$

where k_B and T are the Boltzmann constant and absolute temperature respectively, and Δf is the bandwidth of the measurement. This equation defines the minimum noise of any electrical measurement on a current source with resistance R. It shows that for low noise a high internal resistance of the signal source is required. Solving equation (1) for the resistor R, which is high enough to reach a noise level < 0.2 pA at a bandwidth of 1 kHz (typical for electrophysiological measurements), yields values higher than 400 MOhm. The signal source, however, is not just the patch of membrane (which is very high in resistance), but also any leakage of current between measuring pipette and membrane. When placing a measuring pipette onto a cell surface, one typically observes an increase of resistance between the pipette interior and the surrounding fluid by a factor of 2 to 5, but hardly a complete sealing of the pipette tip (but see below!). Depending on the cleanliness of the exposed cell surface, the geometry, and opening diameter of the pipette one typically ends up with resistance values of 10-20 MΩ - orders of magnitude lower than required for a well-resolved measurement of 1 pA current.

In the period 1973 to 1975 we tried many ways to improve the seal:

- Better cleaning of the muscle surface
- Changing the surface properties of pipettes, rendering them hydrophobic or hydrophilic, or else reversing surface charges
- Optimizing geometry and size of pipettes

Nothing helped, except for making pipettes smaller and smaller in diameter and shaping them in a way that there is a steep cone angle at the tip. Finally, when we arrived at a tip diameter of about 1 μm, we achieved seals on the order of 50 to 100 MΩ, still not optimal, but sufficiently high, such that under optimal conditions characteristic step-like changes in current emerged from the noise. In these experiments, the pipette contained acetylcholine at a very low concentration and was placed near the region, where the nerve terminal had been before its enzymatic removal. For some time, we were in a deadlock situation since the signals were only visible under optimum conditions. Any variation of parameters, which might support the notion that we observe opening and closing of channels, such as lowering voltage, or employing pharmacological blockers would lead to the disappearance of the signals. Finally, around the autumn of 1975, we obtained data with two types of cholinergic agonists (carbamylcholine and suberyldicholine), which showed characteristic differences in the kinetics of the current fluctuations, such that we dared to publish our results (Neher and Sakmann, 1976). The publication was well received by the scientific community and the name 'patch clamp technique' was adopted for this type of measurement, although in hindsight one should have called it 'loose patch clamp'. The 'loose seal' at that time limited the information content and rendered the experiments quite difficult to perform. Therefore, only few laboratories adopted the method in the period 1975-1980.

The Gigaseal

While trying to 'make the best' out of the situation with loose seals, it happened now and then during a measurement, that the seal resistance suddenly increased by orders of magnitude, such that the pipette tip seemed to be completely closed up. It took us some time to realize that this phenomenon was not due to clogging of the tip by some debris but rather provided for what we had aimed at all the time - a perfect seal between pipette and membrane. It resulted in a seal resistance in the Gigaohm range, improving background noise dramatically. By that time three young investigators (Bert Sakmann, myself and Francisco Barrantes) had

been granted laboratory space and small budgets at the Institute to operate our own independent projects next to each other. The opportunity to do independent research at a young age was unique at that time, due to the generosity of our department directors Otto Creutzfeldt, Hans Kuhn and Tom Jovin and the setting was ideal for collaboration, given our backgrounds in Medicine, Physics, and Biochemistry, respectively. Each of us meanwhile had obtained collaborators, such that we could rapidly explore the possibilities provided by the improved measuring conditions. Within a year's time, we established the various measurement configurations, based on the 'tight seal', and published a comprehensive description of the technique (Hamill et al., 1981) together with our collaborators Owen Hamill, Alain Marty and Fred Sigworth. Fred, meanwhile, had succeeded in recording voltage-dependent Na^+-currents from excitable muscle membrane (Sigworth and Neher, 1980), which recapitulated the earlier findings of Hodgkin and Huxley. Together, the data available at that time provided the proof that both electrical and chemical excitation are mediated by ion channels, which open and close in a discrete fashion. This may have been the first time that signals representing conformational changes of single biological macromolecules could be observed in real time.

Surprises Emerging from Tight-Seal Recording

Establishment of the gigaseal not only improved the resolution of the measurement but also made its use very easy. It allowed the potential in the pipette to be changed without invoking forbiddingly large leakage currents. Also, it turned out that the electrically tight seal is also mechanically stable in the sense that the membrane adhered to the glass. This, in turn, allowed us to manipulate patches. Surprisingly we found that one can break the patch by a pulse of suction without losing the seal. This way the cell interior became electrically connected to the pipette and the pipette-cell-assembly remained well isolated against the bath solution. In other words: We had obtained a measurement configuration very similar to standard intracellular recording with which it was possible to measure intracellular voltage changes. Contrary to traditional intracellular recording, however, this way of penetrating the cell membrane was tolerated by very small cells, including mammalian cells in culture. Therefore, this technique, dubbed 'tight seal whole cell recording' or simply 'whole cell recording', was rapidly adopted by many laboratories and largely replaced conventional microelectrode recording in most applications on mammalian cells.

With the availability of a highly sensitive and easy to use technique to study ion channels a number of surprising findings emerged as the result of the work of a large number of laboratories.

- It turned out that ion channels play roles in cell types far beyond those endowed with electrical or chemical excitability. In fact, there is hardly any cell type, which would not express several channel types for volume regulation, mechanosensation or more specialized tasks. Meanwhile, it is known that roughly 200 genes code for ion channels (www.millipore.com/ionchannelome). When we started, we were hoping to find 5 to 10 channel types.

- Ion channels proved to be prime targets for pharmacy. They have large extracellular domains, with which drugs can interact. Blocking or modifying a few channels per cell may influence its entire function. Ion channels and transporters take the second rank among new drug targets after G-protein coupled receptors (Rask-Andersen et al., 2011).

- Mutations in ion channels cause congenital diseases: With 200 genes coding for ion channels, it is not surprising that numerous mutations in channels have been identified, which cause hereditary diseases (Ashcroft, 2000; Lehmann-Horn, 2000). In fact, for many channels, dozens of mutations are known, which lead to different clinical symptoms. While many of these diseases are very rare, research on patient-derived iPS-cells provides unique possibilities to study 'human biology' and to recapitulate altered physiological function of a particular mutation on an otherwise isogenic background (Savla et al., 2014). Such human disease models, although at the single-cell level, should be superior to the widely used rodent models in many aspects.

I would like to point out that most of the 'surprises', discussed here, are not the result of our own work, but based on discoveries by colleagues worldwide. When we started out, our only goal was to prove the ion channel concept. The development since then is an excellent example demonstrating that progress in basic research, driven by curiosity, leads to non-anticipated, surprising results. However, it also shows that it takes decades until such results can be turned into progress of practical biomedical relevance.

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