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ION CHANNELS

TEN YEARS OF PATCH-CLAMP STUDIES

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Ten years ago a revolution in electrophysiology occurred when the improved patch-clamp technique for single-channel and whole-cell current recording was described in such detail and with such clarity [1] that it surprisingly quickly became *the* technique for investigation of the electrical properties of cell membranes. (A simple introduction to patch-clamp electrophysiology and the various configurations used, written for non-specialists, is available [2]). The patch-clamp technique made it possible for the first time to record ionic currents through single channels (pores) in the cell membrane under conditions of complete control over transmembrane voltage and ionic gradients. In the last 10 years biochemical and molecular genetic techniques have also become widely used in the channel field and the character of research in this area has changed profoundly [3].

Although the main excitement in the field of membrane transport has been due to progress in our understanding of ion channels and their functions it should not be forgotten that these pores represent just one class of membrane transport proteins dealing with ion movements. Figure 1 is a simple minimal membrane model that illustrates what could be regarded as the most fundamental transport systems occurring in animal cell membranes. Figure 1 also shows an example of each of the three main types of membrane ion transporters: channels, carriers and pumps. The resting membrane potential in virtually all cells (inside negative with respect to outside) is due to the large transmembrane K^+ gradient and the existence of resting K^+ channels. The transmembrane K^+ gradient is established and maintained by the ATP-driven Na^+,K^+ pump. The transmembrane Na^+ gradient, which results from the operation of the Na^+,K^+ pump together with the existence of the membrane potential, is used as the driving force for concentrative uptake of a number of neutral amino acids. In the steady state K^+ recirculates via the Na^+,K^+ pump and K^+ channel whereas Na^+ recirculates via pump and Na^+-amino acid carrier (co-transporter). Figure 1 illustrates the functional linkage between these three different types of transporter. The most spectacular progress has been in the characterization of channel properties but the patch-clamp technique (whole-cell configuration) has also allowed careful studies of electrogenic carriers such as the neutral Na^+, L-alanine cotransporter to be carried out. In these studies it was possible to establish, for example, that the Na^+/alanine stoichiometric ratio is 1:1 with a very tight coupling [4].

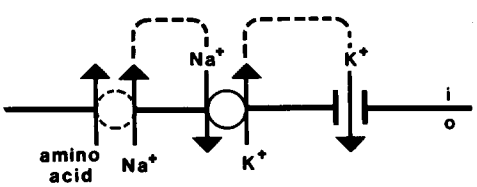

Fig. 1. Simplified diagram for transport of amino acids and cations across the cell membrane. (i) Inner surface of plasma membrane; (O) outer surface of plasma membrane.

Patch-clamp methods have changed radically our ideas about preferred cell types for electrophysiological studies. In the 1950s the success of voltage-clamp experiments, as reviewed by Hodgkin [5], on the squid axon initiated an era in which the study of large cells provided by far the most useful information. Since the 1980s the patch-clamp experiments have been best suited to small round mammalian cells and studies on gland cells have been particularly successful in clarifying aspects of cellular physiology that could not have been investigated with classical intracellular microelectrode techniques [6].

Several classes of ion pore can be distinguished such as gap junctions, ligand-gated channels, voltage-sensitive cation and anion channels, second messenger-gated channels and stretch-sensitive channels. For several but not all of these channels, the genes belonging to separate 'superfamilies' have been characterized [7]. It is important to realize that this type of classification has its problems. Certain ligand-gated channels may, for example, be voltage-sensitive and can in some cases also be modulated by second messengers.

Ion channels have a number of different functions. The classical roles are in the nervous and neuromuscular systems where signal propagation within a cell, Ca^{2+}-dependent transmitter release and muscle contraction are the main functions [8]. In recent years, it has become clear that ion channels also control fluid and electrolyte transport (for example in exocrine glands [9]), regulate hormone secretion (for example insulin secretion [10]), can control lymphocyte functions (for example cytotoxic killing [11]) and in oocytes are involved in fast block of polyspermy [12].

How do ion channels discriminate different ions? Evidently not by size. Even K^+ channels can pass

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ions that are up to 3Å in diameter while Ca^{2+} channels can pass ions that are up to 6Å in diameter. These channels can discriminate very effectively between Na^+,K^+ and Ca^{2+} although these ions are fairly similar in size and are all smaller than 2Å [7]. The generally accepted theory is that ions bind very selectively to their pores. Eisenman's [13] theory of ion selectivity is a first approximation to the solution and considers the trade off in energy that occurs when an ion gives up its waters of hydration in order to bind to a charged site. More recently Eisenman and Alvarez [14] have considered the structure and function of channels and channelogs (channel-like analogues in icosahedral viruses) with the help of computational chemistry.

There has also been considerable progress in our understanding of channel gating, particularly for the voltage-sensitive channels. Channel opening is thought to be controlled by voltage sensors which are charged structures intrinsic to the channel protein. These sensors detect a change in membrane potential, respond to it by moving in the electric field and thereby cause conformational changes that lead to channel opening [15]. When the first sequences for Na^+, Ca^{2+} and K^+ channels became available an unprecedented sequence pattern was noted: the so-called S4 sequence, a structure with basic amino acids (arginine and lysine) at every third or fourth position [16–18]. There is now evidence indicating that this conserved S4 sequence is important for voltage-sensing since mutations in this sequence cause alterations in the voltage-dependence of K^+ channels encoded by the *Shaker* gene of the fruit fly [19].

The progress in our understanding of ion channel properties has not only given new insights into normal cellular functions but has also been important for pathophysiological considerations. In the field of cystic fibrosis (CF*) there have been particularly interesting developments. CF involves a profound reduction of Cl^- permeability in exocrine tissues and for several years now it has been thought that the so-called outwardly rectifying, depolarization-induced Cl^- channel could account for the Cl^- conductance defective in CF. However, recent work shows that CF gene expression is not correlated with rectifying Cl^- channels [20]. Indeed it now seems that this channel is of no particular significance quantitatively in accounting for Cl^- conductance in epithelial cells. This example serves to remind us of the importance of always trying to correlate single-channel current recording with whole-cell current recording in order to estimate to what extent a particular channel type is of quantitative importance to the currents actually flowing across the whole of the cell membrane [2]. In epithelial cells early patch-clamp studies in which single-channel and whole-cell current recordings were compared allowed the first quantification of Ca^{2+}- and voltage-dependent K^+ channels [21].

There has been considerable debate about the properties of the product of the CF gene, the CF transmembrane conductance regulator. It is or is it not the Cl^- channel? Recent work in which expression of the CF gene in non-epithelial invertebrate cells produced a regulated anion conductance [22] supports strongly the possibility that the gene product is itself the regulated Cl^- channel.

The ion channels in the surface cell membrane have been the major focus of attention over the past 10 years but we must not forget that there are also very important ion channels in organelle membranes. Ca^{2+} channels in the sarcoplasmic reticulum of muscle or the endoplasmic reticulum of non-muscle cells play a major role in generating cytoplasmic Ca^{2+} signals that control contraction, secretion, cell division and even cell death. The sarcoplasmic reticulum Ca^{2+} release channel in muscle is now well characterized both biochemically and physiologically [23], and progress is being made in gland cells by combining the use of patch-clamp electrophysiology with that of Ca^{2+}-sensitive fluorescent probes [24]. It is now clear that receptor-activated cytoplasmic Ca^{2+} spiking mediated by inositol trisphosphate is due to the operation of at least two separate intracellular Ca^{2+} channels in the endoplasmic reticulum, one being directly controlled by inositol trisphosphate and the other being activated by Ca^{2+} itself [25].

Channel pharmacology is an area with enormous potential. In recent years, there has been great progress particularly for K^+ channels of which we now have many classes. The insulin-secreting pancreatic β cells provide an interesting example. The dominant resting K^+ channel is sensitive to changes in intracellular ATP and ADP concentration [10], and during glucose stimulation this K^+_{ATP} channel closes causing membrane depolarization and opening of voltage-sensitive Ca^{2+} channels. It is the Ca^{2+} influx through these Ca^{2+} channels that triggers insulin secretion [10]. In non-insulin-dependent diabetes mellitus there is insufficient insulin secretion in response to glucose stimulation but sulphonylurea drugs like tolbutamide or the more potent glibenclamide can markedly enhance secretion [10, 26]. It turns out that tolbutamide and glibenclamide are remarkably selective blockers of the K^+_{ATP} channel, and this explains their therapeutic effect [10, 26, 27]. Interestingly, various sulphonamides act as openers of the K^+_{ATP} channel and one drug in this class, diazoxide, has been used for many years to reduce insulin secretion from insulinomas. Diazoxide and many other K^+ channel openers cause a marked hyperpolarization of the cell membrane in the β cells which, in turn, closes the voltage-sensitive Ca^{2+} channels, explaining the reduction in insulin secretion [27].

This short introduction to the Ion Channel session has obviously not covered all the important developments that have taken place in the last 10 years but I have tried to highlight some interesting trends and hope that some of the information may prove useful as a background for the subsequent presentations.

* Abbreviation: CF, cystic fibrosis.

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