Selectivity filter and conduction pathway biology essay

Science, Biology

Introduction300

Through the way in which they function, opening and shutting their pores in minute fractions of time with incredible sensitivity to voltage or ligand concentration, potassium ion channels fulfil a diverse range of physiological functions. In eukaryotes, potassium channels are integral to the formation of action potentials and neuronal excitability and plasticity. They also have a role in regulating many cellular processes, such as insulin and neurotransmitter release. Potassium channels are important for the preservation of ionic homeostasis in prokaryotes. Different conditions and signals such as Ca2+ and G-proteins are responsible for the activation of the different types of K+ channels and these conditions also correspond to the various molecular subfamilies of the many K+ families that have been found. In this (paper/essay/dissertation?) I aim to summarise the available literature on the Kv1. 3 channel, to provide an up to date report regarding its history, structure and functions, whilst highlighting its significance clinically and otherwise in humans. A 523aa long protein coded for by KCNA3 at the location 1p13. 3, the gene is located amongst the KCNA2 and KCNA10 genes of the same subfamily on chromosome 1. Much like the majority of the Kv1 subfamily genes, with the exception of KCNA7, the KCNA3 gene does not contain introns. The protein coded for by this gene is a voltage-gated, shaker-related subfamily potassium channel protein. The Kv1. 3 channel also belongs to a group of ion channels termed delayed rectifiers, which due to their slow activation kinetics, are important in the control of action potentials. In quiescent T-cells, the Kv1. 3 is the dominant channel involved with maintaining a resting potential, whilst it also forms part of a signalling

cascade that leads to cytokine production and proliferation. The Kv1. 3 channel currently garners great scientific interest for its physiological functions and the scope for its use as a therapeutic target for several autoimmune diseases and several other disorders.

Gene Family and History300

Voltage-gated potassium channels (Kv) are amongst the most structurally and functionally complex voltage-gated ion channels and are also the largest family of potassium channels. This is principally due to a multitude of subunits, their potential to form heterotetramers and the associations with -subunit proteins. Other classes of K+ ion channels include the Ca2+-activated (KCa), inward rectifying (KIR), and two-pore (K2P) channels. These are architecturally and different classes of potassium channels with the number of transmembrane segments in each subunit different for each of the four families. Such disparities are a result of deletions and mutations, mainly to the transmembrane coding regions and as a result of this they have varying functions and roles. Figure - Phylogenetic tree for Kv1-9 families constructed using amino acid sequence alignments through CLUSTALW and analysis by maximum parsimony. The hydrophobic subunits (S1-6) of human Kv channel proteins were used for the analysis {{187 Gutman, George A 2005}}. All K+ channels comprise of a core of -subunits and one or two greatly conserved pore= loop domains, called P-domains. Within this P-domain is the K+ selectivity sequence is common amongst all potassium channels. The shaker gene of the Drosophila was the first cloned Kv channel in 1987 and this instigated the identification of 3more similarly

sequenced genes – shaw, shab and shal – as well as many other voltage- and ligand-gated potassium channels in several organisms {{218 Papazian, Diane M 1987}}. The Kv family can be further divided into multiple subfamilies through function and sequence similarities; Kv1 (shaker-related) , Kv2 (shab-related), Kv3 (shaw-related) , Kv4 (shal-related) and Kv5-12. The Kv5-9 families encode ‘ modifier’ subunits that cannot produce functional channels on their own, but form functional heterotetramers with Kv2 subunits instead. Kv4, 10 and 11 are responsible for coding -subunits that can associate with Kv1 and Kv2 proteins. Comparison of Kv genes in genomic and cDNA libraries, coupled with information from studies using the recently developed patch-clamp technique resulted in the discovery of Kv1. 3, originally termed a type-n K+ channel. A K+ selectivity sequence is a highly conserved sequence (T/SxxTxGxG) found within the pore-loop (P-loop) domain of each subunit of K+ channels. All but one class of K+ channels possess only one p-domain, with two-pore K+ channels being the exception. The eventual characterisation of Kv1. 3

Method of Study 300

Channelomics is a term used for the study of ion channels, such as Kv1. 3, and other membrane channels. Over time these methods have been developed, with a notable breakthroughs occurring in the 1980’s instigating a cascade of rapid discoveries. Due to the nature of ion channels, their study can utilise an array of technologies, from proteomics and biochemistry to biophysics and pharmacology. Several methods of study exist and these allow for the study of ion channels, whether this is at a gene level, a protein

and structural level or perhaps a phenotypic and functional level. Though, it is important to note, methods that work for certain channels, may yield less information on others. At gene and molecular levels, molecular biology techniques, such as RT-PCR and cloning cDNA libraries, can be used to determine sequence characteristics. In fact, (as mentioned earlier) the discovery of Kv1. 3 itself came via screening genomic libraries. This occurred after the molecular characterisation through genetic and molecular research into potassium channel mutants of Drosophila melanogaster, which resulted in the isolation of cDNA coding for the Shaker gene ✔-subunit. (1987?) This resulted in the identification of many Kv channels, within a very short period of time.

Study of Structure

Through identifying a structure, it then becomes possible to put information from other studies into context. The macromolecular nature of ion channels can be studied through the use of light waves. Crystallographic and spectroscopic techniques have had great success, and are an efficient way of obtaining structural information on ion channels. X-ray crystallisation makes it possible to identify the atomic structure of a protein through analysing light refractions. Difficulties that arise whilst working with transmembrane proteins often demand the analysis of fragments of the protein. Studying the structure of ion channels has its difficulties, and the struggle to attain high resolution structures of some membrane proteins provides a barrier in understanding some of their functions.

Study of Function

Stemming from the concept of the voltage-clamp developed by Kenneth Cole, the more highly sensitive patch-clamp technique, established in the 1970’s, allows for the study of an isolated patch of cell membrane and distinct ion channel activity. Patch-clamping records the current that flows directly through individual ion channels, which has been able to attribute certain currents to specific physiological processes. This electrophysiological technique has found itself the most important in channelomics, and can also be used alongside pharmacological interrogation to study the effect on the ion channel activity. More recent developments have seen the use of immunohistochemistry, which allows the localisation of channels in situ through antibodies detecting their complimentary antigens in the channel protein {{220 Vacher, Helene 2008}}. Locating ion channels enables the creation of logical hypotheses and focusing research effectively by linking the abundance of channels in certain locations to their functions. The fundamental role of ion channels in certain physiological functions means that naturally, much of the focus on studying these proteins is at both structural and functional levels, and more importantly, how they affect each other. Such focus has highlighted the importance of the patch clamp technique in ion channel research, and its ability to support genetic information in the understanding of the pathogenesis of a specific ion channel disorder {{219 Jurkat-Rott, K 2004}}. Methods of studying ion channels may also extend to the study of their inhibitors and how they yield an effect on activity. In the case of ion channel kv1. 3, this can be done through a fluorescence systemhttp://www. sciencemag.

org/content/280/5360/69. fullhttp://www. annualreviews. org/doi/full/10. 1146/annurev. physiol. 66. 032102. 113328http://physrev. physiology. org/content/88/4/1407. full. pdf+htmlhttp://www2. montana. edu/cftr/IonChannelPrimers/methods_to_study_ion_channels. htm

Structure and Function 800

http://www. sciencedirect. com/science/article/pii/S0022283610000136The structure of the subunits of the four different classes of K+ channels differs with respect to the way in which they are activated and the number of transmembrane segments (TM) present; 6 TM Kv channels, 4 TM K2P channels, 6/7 TM Kca channels and 2 TM KIR channels. Bearing a striking sequence similarity, the different structures have evolved through the deletions of the transmembrane coding regions of the genes (http://onlinelibrary. wiley. com/doi/10. 1111/j. 1440-1681. 1995. tb02331. x/abstract). Few direct structures of K+ channels are known, including Kv1. 3, though electrophysiological, mutagenesis and molecular cloning studies have inferred similar pore structures across the whole family of channels (http://genomebiology. com/2000/1/4/reviews/0004). The 2TM bacterial KcsA channel has a nearly identical sequence to that of shaker-related channels after the S1-S4 coding sequence (http://www. sciencemag. org/content/280/5360/69. long). The crystal structure of Kv1. 3 has not yet been solved, though the highly homologous Kv1. 2 is thought to have a remarkably similar physical structure. Kv channels exist as tetrameric constructions of similar or matching subunits, the cluster of which forms an ion conducting pore with a four-fold symmetry. An individual ✔-subunit of

Kv1. 3 possesses 6 helical TMs (S1-S6) that form a hydrophobic core. The presence of these TMs was determined via hydropathy analysis and topology studies. (http://www. scopus. com/record/display. url? eid= 2-s2. 0-20444430500vigin= inward&txGid= ACD59C2C983BA4BF3443F676BD3631AC. y7ESLndDIsN8cE7qwvy6w%3a4). The inward most helices are tilted and create the gating region (http://www. sciencemag. org/content/280/5360/69. long). A pore-loop (P-loop) domain is located between transmembrane segments S5 and S6, whilst hydrophilic C- and N- termini protrude outwards into the cytoplasm. Pore-forming contain a single pore-forming region. Low-resolution electron micrographs of purified Kv1. 3 tetramers reveal x–y dimensions of 64 Å.

TM segments

Membrane voltage is a result of the membranes permeability to ions, which is controlled by many ion channels. Membrane voltage regulates itself as for voltage-gated channels, like Kv1. 3, the membrane voltage is what determines whether they remain open. Like other Kv channels, the transmembrane segments S1-S4 represent the voltage sensing domain (VSD) of the channel, whilst the S5 and S6 segments, along with the P-loop domain that connects them, constitute the core region of the channel. Through X-ray analysis, the cytoplasmic portions of the S6 segment from each subunit are seen to cross and form an " inverted teepee" on the intracellular side of the protein, which acts as the channel gate that opens for K+ ions and cradles the selectivity filter (http://www. sciencemag.

org/content/280/5360/69. long). The " inverted teepee" structure is analogous to the closed gate conformation of the ion channel.

Pore-structure

The most essential aspect of the Kv1. 3 structure is the ion conduction pathway, which is found at the centre of the tetramer. A vestibule is formed towards the external entrance of the pore which can be bound to by several inhibitors, some of which are scorpion toxins. Through complementary mutagenesis and thermodynamic mutant cycle analysis, the four of these toxins were shown to exhibit several toxin-channel interactions with a Kv1. 3 channel. The analyses of which enabled the discovery of the vestibule and much about the pore-region’s architecture (http://www. sciencedirect. com/science/article/pii/0896627395901043). The narrowest part of the pore is the 3 Å wide selectivity filter positioned on the extracellular side of the membrane. This extends through ~15 Å towards the middle of the membrane, at which point the pore widens into a ~10 Å diameter spherical water-filled cavity. The " inverted teepee" structure then seals the pore at the intracellular end until it no longer adopts a ‘ closed’ conformation. The water cavity at centre of the channel acts to stabilise the K+ cation in a very hydrophobic location where the energy of the cation would be very high. It does this by surrounding the cation with polarisable water.

Selectivity Filter and Conduction Pathway

Kv channels are highly selective for K+. The selectivity filter present in the P-loop domain is ubiquitous amongst K+ channels is present at the narrowest point of the pore and it determines the cation allowed through the channel.

The P-loop domain along with the S6 helix form the lining of the aqueous pore and are responsible for the high selectivity of the channels (http://www. ncbi. nlm. nih. gov/pubmed/2000495). The function of this selectivity filter has been proven by as filter sequence mutant channels could not discriminate between K+ and other similar ions (http://www. ncbi. nlm. nih. gov/pubmed/8038378? dopt= Abstract&holding= f1000, f1000m, isrctn). The selectivity filter begins as the narrowest 3 Å wide region at the extracellular end of the pore and extends through the membrane for ~15 Å. The wall of the selectivity filter is composed of 12 carbonyl groups, 3 from each subunit, and is highly hydrophilic, unlike the rest of the conduction pathway. It also remains uncharged. 4 binding sites for K+ are present within the selectivity filter. These are defined by 5 rings of oxygen atoms, with each ring made up of one oxygen atom from the carbonyl groups of each subunit. Each K+ interacts with 8 oxygen atoms, after shedding any of its hydrating water molecules, in order to be bound. The oxygen atoms then surround the ion mimicking the spatial arrangement of a hydrated K+ ion. This allows the diffusion of the ion from water at a lower energetic cost of dehydration. As one K+ ion binds to the filter, another one exits through the other side, with an average occupancy of 2 ions at any one time. Potassium ions and water molecules pass through the pathway in an alternate fashion. How such a high affinity is maintained for a specific ion whilst sustaining a high conduction rate is quite perplexing. However, the presence of more than a single ion causes a level of repulsion between the ions whilst the channel only possesses a conductive conformation when there are 2 K+ ions in the filter. This enables a weaker bond with each ion and the adoption of a

conductive conformation as the presence of potassium ions within the filter ensures such a conformation remains stabilised. The pore discriminates Na+ ions from K+ ions because Na+ ions are smaller and bind differently to the oxygen atoms in the filter. This creates an energetically less favourable reaction. When present at high concentrations, Na+ ions can cause the channel to form a ‘ collapsed’ state, when no K+ ions are present in the filter. Good source - http://www. sciencedirect. com/science/article/pii/S0014579303011049

Activation and Gating

The amino acid composition of the helices is integral to the function of the channel. The S4 TM is peculiarly sequenced with every third or four residue an arginine or lysine, which is seen to contribute to the voltage sensing capabilities. The arginine rich segment in S4 (365-371), an acidic residue within S2 (E293) and a negatively charged residue in S3 (D316) have also been shown to contribute to such conformational changes (http://www. ncbi. nlm. nih. gov/pubmed/7605638, http://www. researchgate. net/publication/14536926_Voltage-sensing_residues_in_the_S2_and_S4_segments_of_the_Shaker_K_channel) (http://genomebiology. com/2000/1/4/reviews/0004). At the ~-70mV resting potential, the Kv channel remains closed and membrane depolarisation induces a change in conformation in the VSDs causing the channel gate to open, increasing its ion conductance (http://www. sciencedirect. com/science/article/pii/S0896627303001570). It is extremely unlikely for a shaker-related Kv to stay open at resting potential (http://jgp. rupress.

org/content/114/5/723). Conformational changes by several polar amino acids, called gating charges, in reaction to changing membrane potentials induce gate activity by coupling electrical activity to the mechanics of opening the gate. Several mechanical models of how this happens have been suggested. They differ in how they depict the movement of the S4 membrane, although they seem unified in defining the importance of S4-S5 linker sequences in the coupling mechanism http://www. annualreviews. org/doi/full/10. 1146/annurev. cellbio. 21. 020404. 145837 . The transmembrane potential causes S4 to reposition, allowing more of the positive residues to be exposed to the aqueous exterior of the membrane and move the S4 membrane outward. All the tetramer's positively charged helices move through the membrane, forcing the S6 TM to open the gate by exerting tension on a linker segment found near the end of the S6 TM. As the Kv1. 3 channel belongs to the class of delayed-rectifiers, it is slowly inactivating and remains active for longer, hence its role in forming action potentials. Inactivation occurs after a depolarising change in membrane potential, and it the channel gate does not necessarily have to be shut for an inactive state and that the stimulus may still be present. The way in which Kv1. 3 inactivates is different to that of the ' ball and chain' N-type inactivation of other shaker-related channels, where the N-terminus of one of the subunits clogs the conduction pathway. The Kv1. 3 channel inactivates via a C-type process, a process commonly regarded as a slowly developing and recovering inactivation, though the rate can be influenced by other factors (http://circres. ahajournals. org/content/82/7/739). It involves the conformation of the conserved selectivity filter sequence in a state that

blocks ions from passing through the conduction pathway (http://circres. ahajournals. org/content/82/7/739, http://www. sciencedirect. com/science/article/pii/0896627391903679).

Tetramerisation

These subunits combine to form tetramers, and as previously stated, many heteromeric and homomeric channels can be formed, resulting in a diverse set of possible structures and functions. What is the intrigue about how it folds coming out of the ribosome? http://www. sciencedirect. com/science/article/pii/S0022283610000136 and pretty much every one of its references. A lot of this is being done in kv1. 3...WHY???? ℘ ...I think it is nice to know what is responsible for the correct formation of the protein, how it does it to A protein that is very, very important.

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Reading