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NEURONAL ION CHANNELS AND THEIR SENSITIVITY TO EXTREMELY LOW FREQUENCY WEAK ELECTRIC FIELD EFFECTS

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Abstract — Neuronal ion channels are gated pores whose opening and closing is usually regulated by factors such as voltage or ligands. They are often selectively permeable to ions such as sodium, potassium or calcium. Rapid signalling in neurons requires fast voltage sensitive mechanisms for closing and opening the pore. Anything that interferes with the membrane voltage can alter channel gating and comparatively small changes in the gating properties of a channel can have profound effects. Extremely low frequency electrical or magnetic fields are thought to produce, at most, microvolt changes in neuronal membrane potential. At first sight, such changes in membrane potential seem orders of magnitude too small to significantly influence neuronal signalling. However, in the central nervous system, a number of mechanisms exist which amplify signals. This may allow such small changes in membrane potential to induce significant physiological effects.

INTRODUCTION

Neuronal ion channels are gated pores whose opening and closing may either be intrinsic or else regulated by factors such as voltage or ligands. They are often selectively permeable to particular ions such as sodium (Na), potassium (K), calcium (Ca) or chloride (Cl) ions. They are found in the membranes of all animal cells and play important roles in a number of diverse processes, including nerve and muscle excitation and neurotransmitter and hormone secretion[1]. Any alterations in ion channel function may, therefore, have profound physiological consequences. There are over 400 different genes already identified in the human genome that encode for ion channel proteins.

In this short paper, we will outline the basic properties of neuronal ion channels and show some of the different ways in which their activity can be regulated. We will concentrate on K channels, because the most detailed information is available from work on these channels, but examples will also be described for both Na and Ca channels, where appropriate.

We will then consider how membrane potential changes induced by extremely low frequency (ELF) fields might alter the properties of these channels and, therefore, neuronal excitability. Whilst the changes in membrane potential induced by ELF fields are extremely small, several unique features of the organisation of the central nervous system (CNS) may allow the potential for such small signals to be transduced into observable effects on neuronal signalling.

STRUCTURE AND FUNCTION OF VOLTAGE GATED ION CHANNELS

Rapid signalling in neurons requires fast voltage sensitive mechanisms for closing and opening the pore. A number of such mechanisms exist for voltage gated channels and can be associated with known regions of the proteins. In each case, the degree of activation of a particular mechanism is determined by the membrane potential; consequently, anything that interferes with the membrane voltage can alter channel gating. Best progress in the study of structure–function relationships for ion channels has been made with K channels[2] and most of it from the outstanding X ray crystallography work of R. MacKinnon and his colleagues (see, for example, Reference 3).

Potassium channels

K channels play an important role in a number of different aspects of the electrical responses of the nervous system. K channel activity determines neuronal action potential frequency, shapes the neuronal action potential waveform and controls the strength of synaptic contacts between neurons[1]. Furthermore, K channels have a role in setting (or contributing to) the neuronal resting membrane potential and in regulating the excitability of individual neurons. Since the first molecular cloning of K channel components in the late 1980s, well over 100 different proteins — subunits of distinct types of K channels — have been identified and this number is still growing[4]. Any given neuron may have many different types of K channel expressed in it. For example, the most populous single neuron type in the mammalian brain, the cerebellar granule neuron[5], shows strong levels of expression for at least 26 different K channel alpha subunits[6].

From the sequences of known potassium channel alpha subunits, it is clear that three major superfamilies of K channels can be delineated (Figure 1). They are the six transmembrane domain channels, which are voltage and/or calcium gated; the two transmembrane domain

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inward rectifiers; and the four transmembrane, two-pore domain leak potassium channels.

The six transmembrane domain family is made up of a number of different subfamilies, such as the voltage gated K_V family of channels (K_V1.x to K_V9.x), which are thought to underlie voltage gated delayed rectifier and A-type K channels. KCNQ and EAG channels are also in this family. These are low threshold, voltage gated currents, which are G protein regulated and which often have a non-inactivating component open near the resting membrane potential of the cell. Functionally, in neurons, these channels underlie currents such as the M current. Finally, in this family, are the Slo and SK families of K channel which underlie functionally observed calcium activated K currents. The two transmembrane domain family of inward rectifier K channels (K_{IR} 1.x–7.x) underlie strong inward rectifier currents, inward rectifier currents activated by G protein coupled receptors and, together with sulphonylurea receptors, ATP sensitive K channels. The more recently described four transmembrane, two pore domain K channels (such as TWIK-1, TASK-1 and TREK-1) are thought to underlie leak currents open at all potentials and are expressed heterologously throughout the CNS.

What regions of the channel are important in determining function?

The typical structure of a voltage gated K channel is shown in Figure 2. As stated above, each alpha subunit consists of six transmembrane domains. Four alpha subunits are required for a functional channel. A number of regions of the channel have been identified and related to particular channel functions. For example, the P region (S5–S6 loop) is a hydrophobic hairpin loop, with a highly conserved GYG amino acid sequence which determines the K selectivity of the channel. The unique structural features of this region of the channel allow it to combine exquisite selectivity for K, with high throughput[2].

Other regions of the channel have also been identified as having particular functional roles. The S4 transmembrane segment (Figure 2) conveys voltage sensitivity to the channel[3]. Many amino acid residues in this segment of the channel are positively charged. The N terminus (at least in K_V channels that show fast inactivation) is involved in N type inactivation, and this region is also involved in tetramerisation of the channel.

All of these regions of the channel are sensitive to the membrane field and so, in principle at least, may be influenced by changes in the membrane potential brought about by ELF fields.

Small changes in kinetics can have major consequences

If one simply considers whether or not channels can be activated by changes in the membrane potential, it seems that, at least for the voltage gated K channels, quite large changes in the membrane potential are required. Most classical voltage gated K channels have a threshold membrane potential for activation, which is usually quite positive when compared to the normal resting potential of a neuron (the latter usually is around −70 mV). For example, Figure 3 shows the current–voltage relationship for the mammalian voltage gated K channel, K_V1.1. Expression of K_V1.1 channels gives rise to a delayed rectifier type current, characterised by an increase in membrane current, when the membrane potential is depolarised beyond a certain threshold. These channels inactivate slowly, if at all, and have a role in action potential repolarisation and thus duration. It can be seen that little K_V1.1 current is activated at potentials more negative than −40 to −50 mV, around 20–30 mV more positive than the normal neuronal resting potential of −70 mV.

However, more subtle changes in channel gating may result from relatively small changes in the membrane potential during an action potential and comparatively small changes in the gating properties of a channel can have profound physiological effects. To give just one example, a particular known mutation of the human Na channel β_1 subunit leads to a slight reduction in Na channel inactivation and a prolongation of current through Na channels. The electrophysiological changes

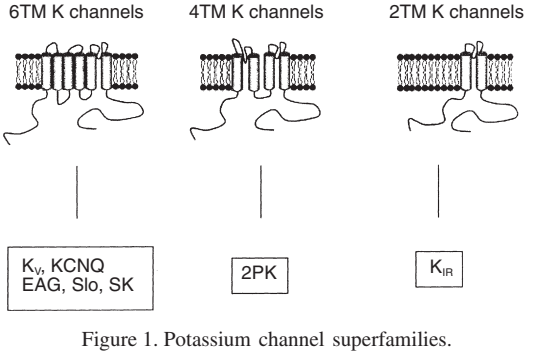

Figure 1. Potassium channel superfamilies.

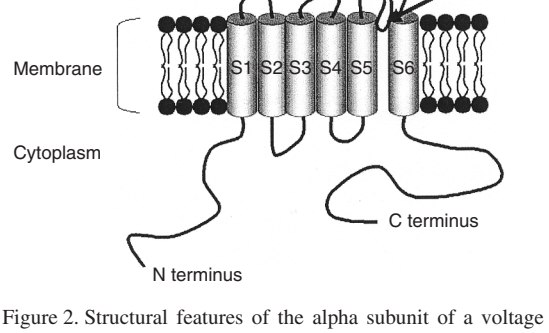

Figure 2. Structural features of the alpha subunit of a voltage gated K channel.

are modest, but the physiological consequences can be large in certain individuals, leading to neuronal hyperexcitability and generalised epilepsy with febrile seizures[7].

Two-pore domain K channels

Recently, a group of K selective pore forming subunits with four transmembrane domains and two pore domains per subunit have been discovered and have been named two-pore domain potassium (2-PK) channels. To date, 15 members of the mammalian 2-PK family have been described (for review, see References 8–10. These subunits are thought to form together as dimers to provide the four P domains required to produce a single functional channel[8]. They can be grouped, loosely, into a number of different classes on the basis of structural and functional properties. TWIK-1 (**t**andem of P domains in a **w**eak-**i**nward-rectifier **K** channel) was the first to be cloned and all subsequent channels have been named in relation to this one[9,10]. A separate nomenclature refers to this channel as KCNK-1[8].

2-PK channels are regulated by a wide variety of neurotransmitters, physiological regulators and drugs, including general anaesthetic agents. For example, TASK-1 and TASK-3 channels are inhibited by extracellular acidification and activation of M_3 muscarinic receptors, whilst current through these channels is enhanced by the general anaesthetic halothane. Currents though TREK-1 channels are enhanced by arachidonic acid, heat, mechanical stretch and many general anaesthetic agents.

Cerebellar granule neurons possess a non-inactivating

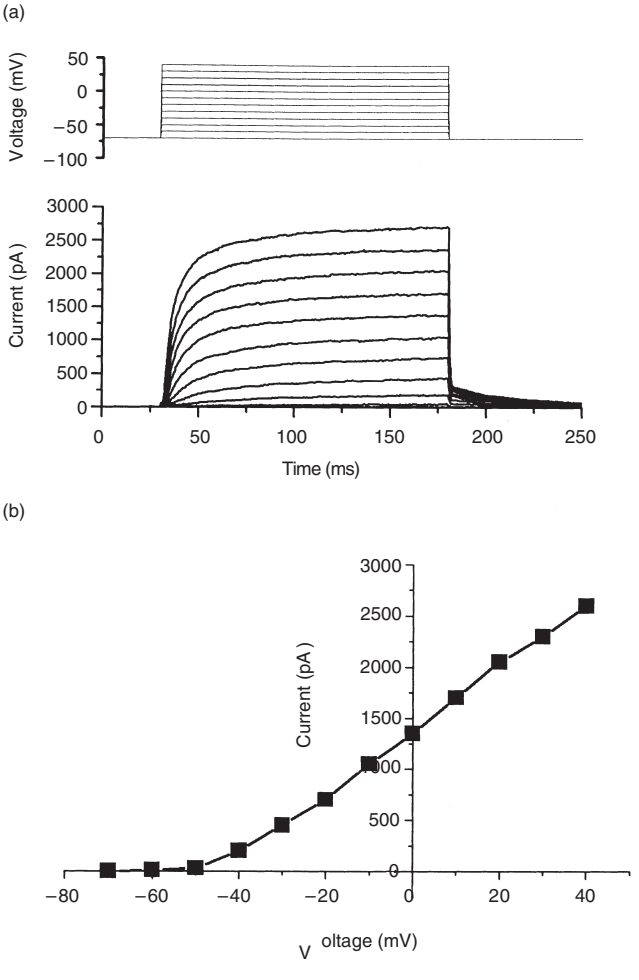

Figure 3. A family of K_V currents. (a) Currents through neuronal voltage gated $mK_V1.1$ channels transfected into CHO cells, following step depolarisations from a holding potential of −70 mV. (b) Current–voltage relationship for the data in (a). The current at the end of the voltage step is plotted against the step potential.

outward potassium current that is active at all membrane potentials. This current, termed IK_{SO} (for standing outward current), which has a major role in regulating the excitability of these cells, is openly rectifying and is inhibited by the activation of muscarinic acetylcholine receptors[11,12]. IK_{SO} is insensitive to the classical K channel blockers tetraethylammonium and 4-aminopyridine, but can be blocked by Ba^{2+}, small extracellular acidification and the endocannabinoid, anandamide. The current is enhanced by the volatile anaesthetic agent halothane. Inhibition of this current by muscarinic receptor activation or extracellular acidification increases granule cell excitability[11,12]. All of the functional properties of IK_{SO} correlate well with those of the 2-PK channels, TASK-1 and TASK-3. This has led to the suggestion that TASK-1, TASK-3, and perhaps TASK-1/TASK-3 heterodimeric channels, underlie IK_{SO}. Thus, IK_{SO} was one of the first currents in native neurons to be identified with the 2-PK channel family.

It is now envisaged that 2-PK channels underlie leak currents found throughout the nervous system. Currents through these channels are open at all potentials, including the resting membrane of neurons (see Figure 4). Thus, unlike K_V channels, any change in membrane potential, however small, will alter their conductance. It follows that anything that alters the activity of 2-PK channels will have a significant effect on neuronal excitability and resting potential.

Regulation of 2-PK channels by biochemical mediators

It is, perhaps, of particular interest here that a number of these 2-PK channels (such as TASK-1, TASK-3 and TREK-1) can also be regulated by phosphorylation through enzymes such as protein kinases A and C[10]. The activity of protein kinase C, for example, can be altered by exposure to extremely low frequency electric fields[13]. Thus, in terms of weak electric field effects on ion channels, not only do we need to consider direct effects on the ion channels themselves but also more subtle effects on intracellular biochemical pathways which may regulate their activity.

ELF FIELD CONSIDERATIONS

An upper limit for the electric field generated by power lines is often regarded as around 5–10 kV m^{-1}. The field dissipates quickly as one moves away from the source and cannot penetrate buildings. Even so, such a field would only generate a change in the membrane potential of neurons in the μV range. Similarly an upper limit for the magnetic field generated by power lines is around 100 μT. This field also dissipates quickly on moving from the source but can penetrate buildings and so its influence will travel further and may be of more importance. Again, however, such a magnetic field has been calculated to induce a potential difference across a membrane or ion channel in the sub-μV range[14]. Such changes are orders of magnitude below the several mV depolarisation required to activate voltage gated channels such as $K_V1.1$, for example. So, this begs the question as to whether there are any circumstances in which such small changes in membrane potential may produce measurable physiological effects.

SIGNAL AMPLIFICATION IN THE CNS

The answer may lie in amplification of the signal. In the CNS, a number of different mechanisms exist, which allow such signal amplification.

Clustering of ion channels

It is known that ion channels cluster in specific regions of a neuron and, in some instances, can be expressed in specific orientations. For example, in axons, Na and K channels cluster at specialised regions of the axon termed nodes of Ranvier[1]. Furthermore, there is a large clustering of Ca channels[15], often in

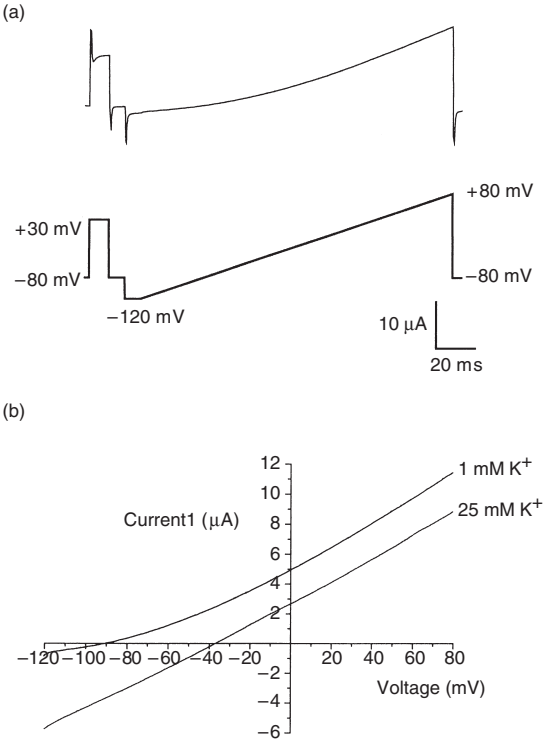

Figure 4. Currents through the 2-PK channel, hTASK-3. (a) Representative current showing current through hTASK-3 channels expressed in *Xenopus* oocytes obtained following a voltage ramp (lower trace) from −120 mV to +80 mV. (b) Current–voltage plot showing that the channel is open at all voltages between −120 and +80 mV and the shift in reversal potential with 25-fold increase in $[K^+]_o$, (C. Clark, unpublished).

linear orientations, at neurotransmitter release sites. Here, the properties of several thousand channels would simultaneously be altered if a change in membrane potential occurred at such a synapse. If, rather than the current *per se*, the concentration of the permeant (in this case Ca) ion is important[14], then there may be significant alterations in the concentration of Ca in the cytoplasm of the cell, particularly just under the Ca channels themselves. Since Ca acts as an intracellular second messenger in neurons, changes in the intracellular concentration of the ion following ion channel opening is often at least as important as any current carried through the channels.

Dendritic spines

In addition to presynaptic clustering of ion channels at transmitter release sites or synapses, postsynaptic clustering of ion channels may be similarly important. Dendritic spines are specialised regions of neurons that process chemical signals from presynaptic axons[16]. They contain clusters of neurotransmitter receptors (such as AMPA and NMDA subtypes of glutamate receptor) and ion channels (such as voltage gated Ca channels). These spines have a small volume, so ion concentrations inside them can alter quickly and significantly. They also have specialised intracellular proteins, which amplify signals from outside the cell. For example, IP_3 receptors and ryanodine receptors mobilise Ca from intracellular stores in response to an influx of Ca from outside the cell. A small increase in intracellular Ca concentration, due to influx of Ca through either NMDA receptors or voltage gated Ca channels, induces larger intracellular Ca increases following mobilisation from stores. Thus, these intracellular proteins give both positive feedback and amplification of the original signal. In the dendritic spines, the combination of small extracellular space and signal amplification means that relatively subtle changes in the activity of Ca channels might have profound effects on intracellular Ca concentration and, therefore, postsynaptic signal processing in the CNS.

Amplification through neuronal networks

Finally, it is possible that very small changes in the excitability of individual neurons may be amplified through neural networks to give much larger functional changes, which can lead to an alteration in physiological responses[17]. Again considering cerebellar granule neurons, axonal projections from these neurons form the parallel fibres of the cerebellum and terminate on the dendrites of Purkinje neurons. It has been estimated that a single Purkinje neuron can receive inputs from 80,000 granule neurons[18]. Thus, very small changes in the electrical properties of an individual granule cell may be amplified many 1000-fold through simultaneous alterations in the many granule cell inputs to Purkinje cells.

SUMMARY

In summary, voltage gated ion channels are regulated in many different ways by voltage and by biochemical mediators — in some cases, rather subtle changes can have large physiological consequences. At first sight, the changes in membrane potential produced by ELF fields seem orders of magnitude too small to significantly alter the properties of ion channels. However, in terms of CNS signalling, two important considerations are clustering of ion channels and signal amplification. These may combine to produce detectable changes in electrical signalling resulting from the initial extremely small changes in membrane potential induced by ELF fields.

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REFERENCES

1. Hille, B. *Ion channels of excitable membranes.* (Sunderland, MA: Sinauer Assoc) (2001).
2. Yellen, G. *The voltage-gated potassium channels and their relatives.* Nature **419**, 35–42 (2002).
3. Jiang, Y., Ruta, V., Chen, J., Lee, A. and MacKinnon, R. *The principle of gating charge movement in a voltage-dependent K^+ channel.* Nature **423**, 42–48 (2003).
4. Coetzee, W. A. and 12 other authors. *Molecular diversity of K channels.* Ann. NY Acad. Sci. **868**, 233–285 (1999).
5. Herrup, K. and Kuemerle, B. *The compartmentalization of the cerebellum.* Ann. Rev. Neurosci. **20** 61–90 (1997).
6. Mathie, A., Clarke, C. E., Ranatunga, K. M. and Veale, E. L. *What are the roles of the many different type of potassium channel expressed in cerebellar granule cells?* The Cerebellum **2**, 11–25 (2003).
7. Wallace, R. H. and 11 other authors. *Febrile seizures and generalized epilepsy associated with a mutation in the Na^+ channel β_1-subunit gene SCN1B.* Nature Genet. **19**, 366–370 (1998).
8. Goldstein, S. A. N., Bockenhauer, D., O'Kelly, I. and Zilberberg, N. *Potassium leak channels and the KCNK family of two-P-domain subunits.* Nature Rev. Neurosci. **2**, 175–184 (2001).
9. Lesage, F. and Lazdunski, M. *Molecular and functional properties of two-pore-domain potassium channels.* Am. J. Physiol. Renal Physiol. **279**, F793–F801 (2001).
10. Lesage, F. *Pharmacology of neuronal background potassium channels.* Neuropharmacology **44**, 1–7 (2003).

11. Watkins, C. S. and Mathie, A. *A non-inactivating K^+ current sensitive to muscarinic receptor activation in rat cultured cerebellar granule neurons.* J. Physiol. **491**, 401–412 (1996).
12. Millar, J. A., Barratt, L., Southan, A. P., Page, K. M., Fyffe, R. E. W., Robertson, B. and Mathie, A. *A functional role for the two-pore domain potassium channel, TASK-1, in cerebellar granule neurons.* Proc. Natl. Acad. Sci. USA **97**, 3614–3618 (2000).
13. Holian, O., Astumian, R. D., Lee, R. C., Reyes, H. M., Attar, B. M. and Walter, R. J. *Protein kinase C activity is altered in HL60 cells exposed to 60 Hz AC electric fields.* Bioelectromagnetics **17**, 504–509 (1996).
14. Galvanovskis, J. and Sandblom, J. *Amplification of electromagnetic signals by ion channels.* Biophy. J. **73**, 3056–3065 (1997).
15. Zenisek, D., Davila, V., Wan, L. and Almers, W. *Imaging calcium entry sites and ribbon structures in two presynaptic cells.* J. Neurosci. **23**, 2538–2548 (2003).
16. Yuste, R., Majewska, A. and Holthoff, K. *From form to function: calcium compartmentalization in dendritic spines.* Nature Neurosci. **3**, 653–659 (2000).
17. Adair, R. K. *Simple neural networks for the amplification and utilization of small changes in neuron firing rates.* Proc. Natl. Acad. Sci. USA **98**, 7253–7258 (2001).
18. Voogd, J. and Glickstein, M. *The anatomy of the cerebellum.* Trends in Neurosci. **21**, 370–375 (1998).