In response to free polyunsaturated fatty acids (PUFAs), voltage-gated K channels open at more negative membrane voltages. This opening could reduce cellular excitability and be beneficial for patients with epilepsy and cardiac arrhythmia. However, under normal physiological conditions a large amount of PUFA binds to albumin, the most abundant protein in blood plasma (~640 μM), also present in the cerebrospinal fluid (~3 μM). We therefore sought to examine to which extent albumin interfered with the action of docosahexaenoic acid (DHA; the most common n-3 PUFA in the brain) on a voltage-gated K channel. We expressed a mutated Shaker K channel, supersensitive to DHA, in Xenopus oocytes and investigated the K current using the two-electrode voltage-clamp technique. 21 μM DHA, in the absence of albumin, shifted the voltage dependence of the K channel conductance −15 mV along the voltage axis. 0.6 μM albumin reduced the DHA-induced shift by 50%. Thus, in cerebrospinal fluid a significant amount of DHA is expected to be free - not bound to albumin - and possible to directly interact with ion channels. Calculations suggested that each albumin molecule can bind up to seven DHA molecules.

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Construction of a K Channel Hypersensitive to Polyunsaturated Fatty Acids

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Negatively charged polyunsaturated fatty acids (PUFAs) modulate the voltage dependence of several voltage-gated ion channels. The PUFAs are probably incorporated into the cell membrane close to the channel's voltage sensors wherein an electrostatic modulation occurs. The interaction site does not overlap with other known binding sites for toxins and drugs. For voltage-gated K channels, the PUFAs increase the open probability at negative membrane voltages thereby being potent modifiers of cellular excitability. However, in spite of the desirable effect of the PUFAs, they are probably no good pharmaceutical candidates due to their biological promiscuity. To find potent, and more specific small-molecule compounds, more knowledge about the modulation is needed. Our goal was to construct a K channel with very high sensitivity to PUFAs. We expressed the ShakerIR K channel in Xenopus oocytes and studied the ion currents with a two-electrode voltage clamp technique. Positive charges at the extracellular end of the voltage sensor S4 are expected to potentiate the effect of negatively charged PUFA molecules. Therefore we systematically introduced arginines, one by one, at long stretch of S4. The mutations with the largest effects were then combined in different ways. The most effective mutation, with respect to PUFA-induced shifts of the channel's voltage dependence, contained two extra arginines outside the positive charges of the wild-type S4. Introducing these two positively charged residues in combination with an altered pH from 7.4 to 9.0 increased the current about 100 times at negative voltages. This supersensitive channel can be instrumental in the search for medical drugs against hyperexcitability diseases such as cardiac arrhythmia, epilepsy, and pain.

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Sphingomyelinase D activates Native Voltage-Gated Ion Channels in Mammalian Cells

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Sphingomyelinase D (SMase D), a phospholipase present in the venom of Loxoceles spiders and also expressed by certain pathogenic bacteria, cleaves the choline headgroup from sphingomyelin. In Xenopus oocytes, SMase D treatment enables voltage sensors to mobilize at more negative potentials, consequently activating voltage-gated ion channels at more hyperpolarized voltages. If this effect is not an idiosyncrasy of the sphingomyelin-rich oocyte membrane or a function of aberrant membrane trafficking of over-expressed channels, SMase D may permit control of voltage-gated ion channel activity in non-excitable cells (e.g. lymphocytes). Hence, we tested the effect of SMase D on voltage-gated potassium (Kv) channels in mammalian cells. SMase D treatment left-shifts the conductance-voltage (G-V) relation of Kv channels heterologously expressed in CHO cells, resembling its effect in oocytes. Furthermore, SMase D also shifts the G-V curve of endogenously expressed Jurkat T-lymphocyte Kv1.3 channels in the hyperpolarized direction, activating channels at hyperpolarized potentials where they otherwise remain largely deactivated. Conceivably, modification of membrane lipids via either a physiological or pathological means can control or alter activity of voltage-gated ion channels.

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Dual Effect of PIP_2 on *Shaker* K^+ Channels

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Phosphatidylinositol-(4,5)-bisphosphate (PIP_2) is a phospholipid of the plasma membrane that has been shown to be a key regulator of several ion channels. Functional studies and more recently structural studies of Kir channels have revealed the major impact of PIP_2 on the open state stabilization. A similar effect of PIP_2 on the delayed rectifiers Kv7.1 and Kv11.1, two voltage-gated K^+ channels, has been suggested, but the molecular mechanism remains elusive. By combining giant-patch ionic and gating current recordings in COS-7 cells, and voltage-clamp fluorimetry in Xenopus Oocytes, both heterologously expressing the voltage-dependent Shaker channel, we show that PIP_2 exerts (1) a gain-of-function effect on the maximal current amplitude, consistent with a stabilization of the open state and (2) a loss-of-function effect by positive-shifting the activation voltage dependence, most likely through a direct effect on the voltage sensor movement, as illustrated by Molecular Dynamics simulations.

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Modulation of K_v and K_{ir} Currents by 15-Epi-Lipoxin-A4 in activated Macrophages. Implications for the Regulation of the Innate Immune Response

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Potassium channels play a pivotal role in the modulation of macrophage physiology. Blockade of voltage dependent potassium channels (K_v) channels by specific antagonists decreases macrophage cytokine production and inhibits proliferation. In the presence of aspirin, acetylated COX-2 loses activity required to form prostaglandin but maintains oxygenase activity to produce 15*R*-HETE from arachidonate. This intermediate product is transformed via 5-LOX into epimeric lipoxins, termed 15-epi-lipoxins (e-LXA_4). K_v channels have been proposed as anti-inflammatory targets. Therefore, we studied the effects of e-LXA_4 on early signaling and on K_v and inward rectifier potassium channels (K_{ir}) in bone marrow-derived macrophages (BMDM). Experiments were performed in BMDM cells and electrophysiological recordings were performed by the whole-cell patch-clamp technique. Treatment of BMDM with e-LXA_4 inhibited LPS-dependent activation of NF-κB and IKKβ activity, at the same time that protected against LPS activation-dependent apoptosis. In addition, acute treatment of LPS-stimulated BMDM cells with e-LXA_4 resulted in a decrease of K_v currents, compatible with attenuation of the inflammatory response. More importantly, long-term treatment of LPS-stimulated BMDM with e-LXA_4 significantly reverted LPS effects on K_v and K_{ir} currents. These effects were mediated, at least in part, via the lipoxin receptor (ALX), since were partially reverted in the presence of a selective ALX receptor antagonist. We provide evidence for a new mechanism by which e-LXA_4 contributes to inflammation resolution consisting in the reversion of LPS effects on K_v and K_{ir} currents in macrophages.

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Caveolin-1 Regulates Kir2.1 Channel activity through Interaction with Cholesterol

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A growing number of studies show that different types of ion channels are regulated by the level of membrane cholesterol and localize in caveolae. It has been proposed that cholesterol-induced regulation of ion channels might be attributed to partitioning into caveolae and association with caveolin-1 (Cav-1). We tested, therefore, whether Cav-1 is required for cholesterol sensitivity of Kir2.1 channels. Our observations show that Cav-1 co-precipitates with Kir2.1 channels and that co-expression of Kir2 channels with Cav-1 in HEK293 cells, a cell line with very low levels of endogenous caveolin, results in suppression of Kir2 activity indicating that Cav-1 is a negative regulator of Kir2 function. These observations are confirmed by comparing Kir currents in