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Axonal Kv7.2/7.3 channels

Caught in the act

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Neurons target high densities of fast voltage-gated Na^+ (Nav) and K^+ (Kv) channels to their axonal initial segment (AISs) and nodes of Ranvier, subcellular domains governing the initiation and conduction of fast action potentials, respectively. Kv1 and Kv3 channels open rapidly to repolarize the action potential. In contrast, Kv7 channels, underlying the M-current (or I_M),[1] are characterized by slow activation and de-activation kinetics leading to outward K^+ flow lasting for many milliseconds after the action potential, providing an ionic mechanism for adjusting the action potential firing rates.[1-3] A decade of work,[4] including immunohistochemical, cell biological, and electrophysiological studies using somatic recordings, have provided evidence that the slow-gating Kv7 channels are also partners with Nav channels at the AISs and nodes.[2,5-8] In vertebrates, the Kv7 channel subfamily consists of five subunits (Kv7.1 – 7.5). Neuronal Kv7.2 subunits are expressed either as homotetramers or as heterotetramers with Kv7.3 subunits.[1] Kv7.2 and Kv7.3 share a conserved domain in the C-terminus for binding to Ankyrin G, thus increasing surface expression of Kv7.2 and Kv7.3 to the AIS and nodes of Ranvier.[6,7] Despite a wealth of studies focusing on their modulation and pathogenic mutations, the precise gating kinetics of Kv7.2/7.3 channels in the central nervous system axonal domains where they are natively expressed remained unknown (but see[7]). Here, we summarize key findings from a recent study,[4] in which we directly measured for the first time the neocortical axonal M-current with patch-clamp recording.

In myelinated axons, the known potassium currents activated in the same voltage range as I_M are limited to AIS and juxtaparanodal Kv1 and nodal Kv3 subfamily channels, both of which can be selectively blocked by 4-aminopyridine (4-AP). Under these conditions, activation and deactivation voltage-clamp step protocols could be used across a large voltage range (–120 mV to +45 mV), allowing a complete examination of whole-axon currents evoked at the cut-end of myelinated axons. The outward K^+ currents were activated with a time constant (τ) of ~40 ms (at –40 mV) and a Boltzmann fit to the normalized conductance revealed a half-maximum activation at –34 mV together with a slope factor of 8.6 mV. Consistent with the presumed role of Kv7.2/7.3 channels, the outward currents were blocked with the Kv7 selective blocker XE-991 (ref.[1]). Different stoichiometric combinations of Kv7.2 – 7.5 subunits have been reported to generate functionally diverse M-currents.[1,3] The various Kv7 channels differ in sensitivity to block by extracellular tetraethyl ammonium chloride (TEA), allowing this compound to be used to determine the subunit composition of the axonal, 4-AP insensitive and XE-991 sensitive current.[1,3] The fit with a single-power Hill equation showed that the IC_{50} for TEA block was approximately 3 mM, indicating that the axonal channels were formed by heteromeric assembly of Kv7.2 and Kv7.3 subunits.[5] This observation was in good agreement with the confocal

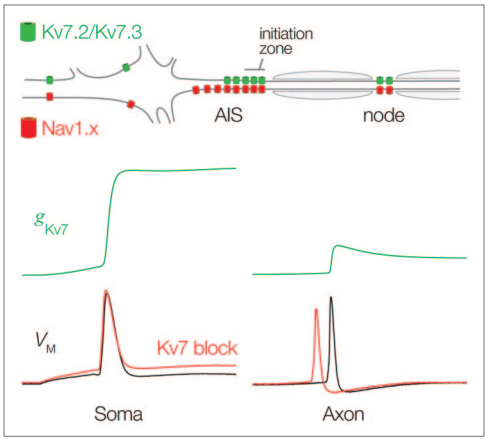

Figure 1. Kv7.2/7.3 channel density estimations revealed on average ~1 channel/μm² in the somatodendritic membrane and up to 10 channels/μm² in distal AIS sites, near the action potential initiation zone (~40-fold lower compared with the local Nav channel density[5]). Broad somatic action potentials efficiently activate Kv7, based on the Kv7.2/7.3 conductance model (green) and experimentally observed Kv7 channel block with XE-991 (red). In contrast, narrower nodal action potentials are sensitive to the resting membrane depolarization after XE-991, causing inactivation of Nav channels and a reduction in action potential amplitude.

imaging of antibody labeling of the same axons, showing that both Kv7.2 and Kv7.3 subunits could be detected and were highly co-localized at all neocortical axonal initial segments and nodes of Ranvier examined. Finally, direct cell-attached and outside-out recordings from the axon initial segments showed that M-channels at the most distal end of the AIS were present at a density of ~10 μm^{-2} with gating properties similar to the distal axon.

What could be the role of M-current in the nodal domains? Computational modeling, constrained by the experimentally observed M-current data, showed that due to the small local capacitance and Kv1 activation in myelinated axons, the axonal action potentials are very narrow (~350 μs half-width) and the slow mono-exponentially activating Kv7 channels (e.g., ~20 ms at 0 mV, 35 °C) are only ~2% recruited by a single axonal action potential (**Fig. 1**). These predictions were corroborated with XE-991 blocking experiments that did not change the axonal action potential after-depolarization. But since Kv7.2/7.3 channels are 4% open at the resting potential XE-991 also depolarized the resting membrane potential of the axon, reduced nodal Nav channel availability, and thereby diminished the amplitude of the action potential (**Fig. 1**).

In summary, a combination of targeted subcellular voltage-clamp and imaging approaches captured for the first time the kinetics and density of Kv7.2/7.3 heterotetramers in their native axonal domains. Kv7.2/7.3 channels in axons share many of the biophysical properties with channels studied in earlier work using heterologous expression systems.[3] Although the similarity in gating is re-assuring, the experimental work shows that the impact of Kv7.2/7.3 activation is highly diverse and depends on subcellular location. Slow voltage fluctuations, such as synaptic potentials activating Kv7.2/7.3 efficiently in the subthreshold range, propagate only to a limited extent into proximal regions of the axon. Kv7.2/7.3 expression in nodal domains mostly influenced the Nav channel availability. Recently, missense mutations in the Kv7.2 ion pore, voltage sensor or the C-terminus that cause severe epilepsy and developmental delay (i.e., epileptic encephalopathy) have been shown to reduce currents strongly.[9] While these mutations likely increase excitability in the peri-somatic regions, they may paradoxically diminish the excitability of the nodes. The increased capabilities to record ion channel gating and excitability in specific axonal domains[5] allow such predictions to be tested more directly.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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