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The MiRP2-Kv3.4 potassium channel: muscling in on Alzheimer's disease

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Running title: MiRP2-Kv3.4 in Alzheimer's disease

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Abstract

In this issue of *Molecular Pharmacology* (p xxx), Pannacione *et al* provide evidence of a role for the voltage-gated potassium channel α subunit Kv3.4 and its ancillary subunit MiRP2 in A β peptide-mediated neuronal death. The MiRP2-Kv3.4 channel complex - previously found to be important in skeletal myocyte physiology - is now argued to be a molecular correlate of the transient outward potassium current upregulated by A β peptide, considered a significant step in the etiology of Alzheimer's disease. The authors conclude that MiRP2 and Kv3.4 are upregulated by A β peptide in an NF κ B-dependent fashion at the transcriptional level, and the sea anemone toxin BDS-I is shown to protect against A β peptide-mediated cell death by specific blockade of Kv3.4-generated current. The findings lend weight to the premise that specific channels such as MiRP2-Kv3.4 could hold promise as future therapeutic targets in Alzheimer's disease and potentially other neurodegenerative disorders.

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The etiology of Alzheimer's disease (AD) remains unknown. Pathological hallmarks include cortical neuron loss, proteinaceous senile plaques, and neurofibrillary tangles. According to the β -amyloid (or $A\beta$) hypothesis, $A\beta$ peptide - the main constituent of plaques - is considered to be linked to the selective neurodegeneration seen in AD. Although much progress has been made in characterizing $A\beta$'s neurotoxic effects, the mechanism by which $A\beta$ induces neuronal death is still unknown (Pallas and Camins, 2006).

Potassium ion (K^+) efflux is a critical step in the apoptosis of many cell types in response to a range of pro-apoptotic stimuli (Yu, 2003). Recent evidence points to an increase in voltage-gated potassium (Kv) channel current as underlying $A\beta$ -induced neuronal apoptosis; however, the molecular mechanism by which K^+ current is upregulated and the subunit identity of the Kv channels involved are still under debate (see below). Kv channel pore-forming (α) subunits are generated by a numerous and functionally diverse family of genes, with ~40 known members in the human genome. Each α subunit contains six transmembrane domains, a voltage sensor and pore elements; four α subunits co-assemble to form the functional tetramer. *In vivo*, Kv channels are each regulated by a host of possible non pore-forming subunits with either cytoplasmic or transmembrane disposition; some are regulatory proteins that also modulate other protein types; others are dedicated Kv channel ancillary subunits (McCrossan and Abbott, 2004).

While mutations in several Kv channel α and ancillary subunit genes are associated with inherited human diseases – referred to as 'channelopathies' – the massive number of different Kv channel sub-types that can potentially arise from the matrix of possible α - α and α -ancillary subunit interactions has thus far hampered identification of the precise molecular entities in the large majority of both physiological Kv currents and suspected Kv channel-related disorders.

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AD is no exception. Ramsden and colleagues (Ramsden et al., 2001) noted that long-term exposure of rat cerebellar granule neurons to unaggregated A β ₁₋₄₀ led to an increase in both the inactivating I_{KA} current (peak-end current) and I_{KV} (end current) while acute administration had no effect. Increased current was also observed using a protocol employing a -50 mV prepulse, which removes the majority of the I_{KA} current component. These data suggested that A β does not increase K⁺ current through direct modulation of the potassium channel itself, but instead may work at the level of transcription, translation or membrane targeting of the potassium channel α subunits and/or ancillary β subunits.

In a follow-up study by the same group (Plant et al., 2006), it was demonstrated that increases in I_{KA} current density, and expression of Kv4.2 and Kv4.3, in response to A β ₁₋₄₀ and A β ₁₋₄₂ were temporally related. Effects could be prevented by cycloheximide and brefeldin A, indicative of a mechanism involving protein expression and trafficking rather than degradation or direct interaction with mature channels at the surface; furthermore these effects were reproducible *in vitro*. Another group noted similar effects after isolating rat cerebral cortex and hippocampi following an intracerebroventricular injection of A β ₂₅₋₃₅ (Pan et al., 2004). Quantifying Kv1.4, 1.5, 2.1, 4.2, and 4.3 mRNA levels using RT-PCR, they found increases in Kv1.4, 2.1, and 4.2 message, confirmed at the protein level by immunoblotting. While results of these studies were compelling, and both indicated increased expression of several channels following A β exposure, evidence was still lacking regarding the absolute molecular identity of the channels coordinating increased potassium current in response to A β .

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Angulo and colleagues employed an alternative strategy to provide some insight into the identity of the potassium channel subunits potentially involved in the neurodegeneration that is characteristic of AD (Angulo et al., 2004). Using an unbiased approach, they examined by microarray analysis which genes were differentially expressed in RNA isolated from the frontal cortex of patients demonstrating either of two Braak stages of the disease (AD I-II vs AD V). *KCNC4* (GenBank #M64676), which encodes the Kv3.4 α subunit, was the only gene with reportedly increased expression in both AD stages compared to age-matched control samples. Furthermore, increases in expression correlated with disease severity. RT-PCR and immunoblotting confirmed the increase in expression of Kv3.4 in AD; however, the mechanism by which this occurred and its contribution to the neurodegenerative process were not extensively studied.

In an article in this issue of *Molecular Pharmacology*, Pannacione *et al* shed further light on the matter. They show that exposure of NGF-treated PC12 cells and rat hippocampal neurons to A β ₁₋₄₂ leads to an increase in the transcription of Kv3.4 mRNA, and subsequent translation and membrane insertion of Kv3.4 protein. This is mediated through an NF κ B-dependent pathway; inhibition of NF κ B both prevents the increased expression of Kv3.4 and protects against the neurotoxic effects of A β . The authors also examine the effects of blockade of the Kv3.4 channel using the Kv3 subfamily-specific sea anemone toxin BDS-I, finding that this is also protective against A β neurotoxicity. Additionally, the authors note an increase in mRNA and protein levels of MiRP2 (encoded by the *KCNE3* gene), an ancillary subunit of several Kv channels including Kv3.4.

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MiRP2-Kv3.4 potassium channels were previously found essential for normal function of skeletal myocytes (Abbott et al., 2001). Homomeric Kv3.4 (originally designated Raw3) channels pass suprathreshold-activating K^+ -selective currents, fast-inactivating by virtue of a cytoplasmic inactivation domain that plugs the intracellular vestibule of the pore upon activation (Schroter et al., 1991; Vega-Saenz de Miera et al., 1992). Co-assembly with MiRP2 shifts the voltage-dependence of Kv3.4 activation by -45 mV, converting the channel to a subthreshold-activating channel that contributes to skeletal muscle resting potential. An inherited mutation (R83H) in the cytoplasmic domain of human MiRP2 disrupts the ability of this ancillary subunit to shift the voltage dependence of Kv3.4 and thus its ability to contribute to myocyte resting potential. This is associated with periodic paralysis, a channelopathy characterized by skeletal muscle dysfunction, leading to attacks of paralysis range from hours to days (Abbott et al., 2001). The MiRP2-Kv3.4 complex is highly regulated by protein kinase C – phosphorylation of the Kv3.4 inactivation domain disrupts its ordered structure and its ability to inactivate the channel (Antz et al., 1999); constitutive phosphorylation of serine 82 on MiRP2 is required for its modulatory effects on Kv3.4 (Abbott et al., 2006). Thus, global dephosphorylation of MiRP2 and Kv3.4 reduces current by positive-shifting activation and speeding inactivation; whilst when phosphorylated the channel complex can activate at more negative voltages and inactivates much more slowly, increasing overall current.

In summary, the findings by Pannaccione and colleagues suggest that Kv3.4 – perhaps in complexes with MiRP2 – contributes to the pathogenesis of AD and that targeting this channel may be a new therapeutic strategy for the treatment of the disease. Still, questions remain unanswered that may be the topic of future studies directed at further understanding the role of MiRP2 and Kv3.4 in neuronal death and AD (Figure 1). First, Pannaccione *et al* demonstrate that both subunits are upregulated by $A\beta$, but do they co-assemble in this context and/or is

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MiRP2 perhaps performing roles in AD pathology other than the suggested one of regulating Kv3.4? MiRP2 is a promiscuous Kv channel ancillary subunit, and is also known to regulate delayed rectifier Kv channels Kv2.1 and Kv3.1 in PC12 cells (McCrossan et al., 2003), the latter of which can also form heteromeric complexes with Kv3.4 in some neurons (Baranauskas et al., 2003). Second, is the MiRP2-Kv3.4 complex regulated by PKC in neurons, either physiologically or pathophysiologically, and if so could interference with this regulation also provide potential therapeutic avenues? Third, is it the current at highly depolarized voltages or at subthreshold voltages that is most important for A β -related cell death? This is potentially highly significant and relates to the previous points: PKC-phosphorylated MiRP2 increases Kv3.4 current 25-50% at strongly depolarized voltages due to an increase in unitary conductance, but increases current by 2 orders of magnitude at -40 mV due to greatly increased open probability. Fourth, and lastly, if MiRP2-Kv3.4 is targeted for AD therapy, what would be the consequences for other tissues requiring this channel, such as skeletal muscle?

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Footnotes

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Figure legend

Figure 1. Proposed mechanism and some questions remaining in the roles of MiRP2 and Kv3.4 subunits in apoptosis in AD.

