MOL PHARM

MOLECULAR PHARMACOLOGY

PERSPECTIVE

The MiRP2-Kv3.4 Potassium Channel: Muscling In on Alzheimer's Disease

Eun Choi and Geoffrey W. Abbott

Greenberg Division of Cardiology, Department of Medicine and Department of Pharmacology, Cornell University, Weill Medical College, New York, New York

ABSTRACT

In this issue of *Molecular Pharmacology* (p. 665), Pannacione et al. provide evidence of a role for the voltage-gated potassium channel α subunit Kv3.4 and its ancillary subunit MiRP2 in β-amyloid (Aβ) 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 up-regulated by Aβ peptide, considered a significant step in the etiology of Alzheimer's disease. The authors conclude that MiRP2 and Kv3.4 are up-regulated by Aβ peptide in a nuclear factor κB-dependent fashion at the transcriptional level, and the sea anemone toxin BDS-I is shown to protect against Aβ 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.

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 Aβ) hypothesis, Aβ 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 the neurotoxic effects of Aβ, the mechanism by which it induces neuronal death remains unknown (Pallàs and Camins, 2006).

Potassium ion (K$^+$) efflux is a critical step in the apoptosis of many cell types in response to a range of proapoptotic stimuli (Yu, 2003). Recent evidence points to an increase in voltage-gated potassium (Kv) channel current in the etiology of Aβ-induced neuronal apoptosis; however, the molecular mechanism by which K$^+$ current is up-regulated 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 coassemble 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).

Although 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 subtypes 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.

AD is no exception. Ramsden et al. (2001) noted that long-term exposure of rat cerebellar granule neurons to unaggregated Aβ_{1-40} led to an increase in both the inactivating I_{KA} current (peak-end current) and I_{KV} (end current), whereas 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.

ABBREVIATIONS: AD, Alzheimer's disease; Aβ, β-amyloid.

These data suggested that Aβ does not increase K$^+$ current through direct modulation of the potassium channel itself but instead might 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 Aβ_{1-40} and Aβ_{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 after an intracerebroventricular injection of Aβ_{25-35} (Pan et al., 2004). Quantifying Kv1.4, 1.5, 2.1, 4.2, and 4.3 mRNA levels using reverse transcription-polymerase chain reaction, they found increases in Kv1.4, 2.1, and 4.2 message, confirmed at the protein level by immunoblotting. Although results of these studies were compelling and both indicated increased expression of several channels after Aβ exposure, evidence was still lacking regarding the absolute molecular identity of the channels coordinating increased potassium current in response to Aβ.

Angulo et al. (2004) 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. 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 versus AD V). *KCNC4* (GenBank accession number M64676), which encodes the Kv3.4 α subunit, was the only gene with reportedly increased expression in both AD stages compared with age-matched control samples. Furthermore, increases in expression correlated with disease severity. Reverse transcription-polymerase chain reaction 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. (2007) shed further light on the matter. They show that exposure of nerve growth factor-treated PC-12 cells and rat hippocampal neurons to Aβ_{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 a nuclear factor κB-dependent pathway; inhibition of nuclear factor κB both prevents the increased expression of Kv3.4 and protects against the neurotoxic effects of Aβ. 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 Aβ neurotoxicity. In addition, 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.

MiRP2-Kv3.4 potassium channels were previously found to be 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 (Schröter et al., 1991; Vega-Saenz de Miera et al., 1992). Coassembly 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
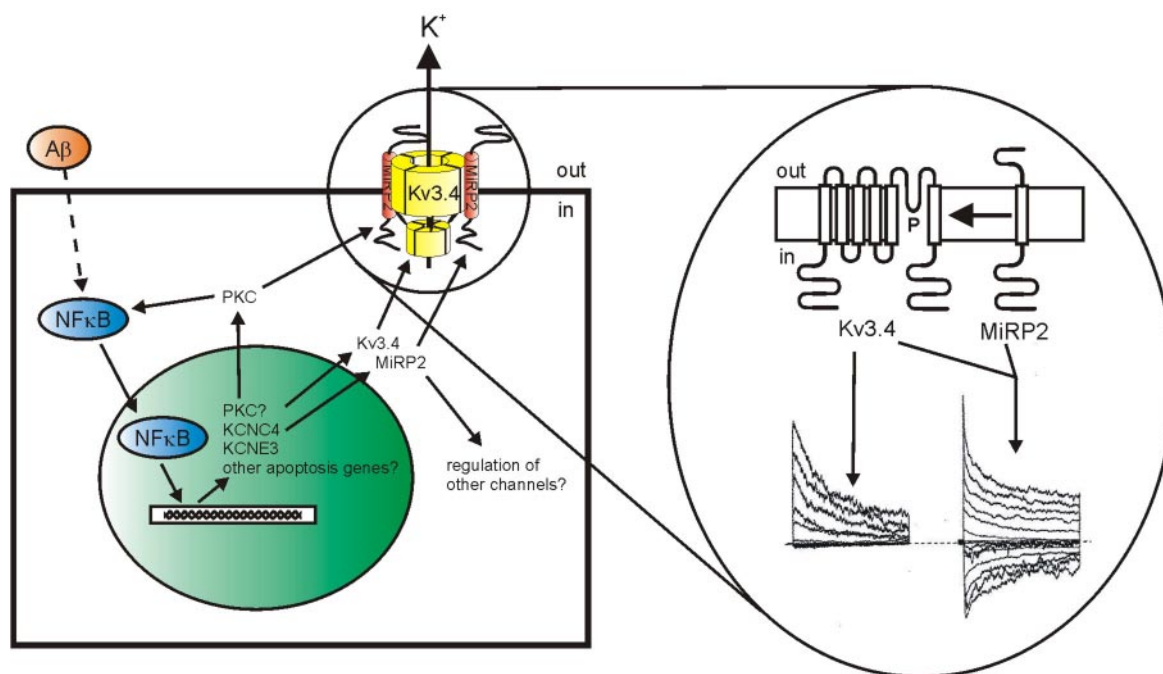

Fig. 1. Proposed mechanism and some questions remaining in the roles of MiRP2 and Kv3.4 subunits in apoptosis in AD.

paralysis, a channelopathy characterized by skeletal muscle dysfunction, leading to attacks of paralysis lasting 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; 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 et al. (2007) 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 (Fig. 1). First, Pannaccione et al. (2007) demonstrate that both subunits are up-regulated by Aβ, but do they coassemble in this context and/or is MiRP2 perhaps performing roles in AD pathologic lesions 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? 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 Aβ-related cell death? This is potentially highly significant and relates to the previous points: PKC-phosphorylated MiRP2 increases Kv3.4 current 25 to 50% at strongly depolarized voltages because of an increase in unitary conductance, but increases current by 2 orders of magnitude at -40 mV as a result of greatly increased open probability. Fourth, and last, if MiRP2-Kv3.4 is targeted for AD therapy, what would be the consequences for other tissues requiring this channel, such as skeletal muscle?

References

Abbott GW, Butler MH, Bendahhou S, Dalakas MC, Ptacek LJ, and Goldstein SA (2001) MiRP2 forms potassium channels in skeletal muscle with Kv3.4 and is associated with periodic paralysis. *Cell* **104:**217–231.

Abbott GW, Butler MH, and Goldstein SA (2006) Phosphorylation and protonation of neighboring MiRP2 sites: function and pathophysiology of MiRP2-Kv3.4 potassium channels in periodic paralysis. *Faseb J* **20:**293–301.

Angulo E, Noe V, Casado V, Mallol J, Gomez-Isla T, Lluis C, Ferrer I, Ciudad CJ, and Franco R (2004) Up-regulation of the Kv3.4 potassium channel subunit in early stages of Alzheimer's disease. *J Neurochem* **91:**547–557.

Antz C, Bauer T, Kalbacher H, Frank R, Covarrubias M, Kalbitzer HR, Ruppersberg JP, Baukrowitz T, and Fakler B (1999) Control of K+ channel gating by protein phosphorylation: structural switches of the inactivation gate. *Nat Struct Biol* **6:**146–150.

Baranauskas G, Tkatch T, Nagata K, Yeh JZ, and Surmeier DJ (2003) Kv3.4 subunits enhance the repolarizing efficiency of Kv3.1 channels in fast-spiking neurons. *Nat Neurosci* **6:**258–266.

McCrossan ZA, and Abbott GW (2004) The MinK-Related Peptides. *Neuropharmacology* **47:**787–821.

McCrossan ZA, Lewis A, Panaghie G, Jordan PN, Christini DJ, Lerner DJ, and Abbott GW (2003) MinK-related peptide 2 modulates Kv2.1 and Kv3.1 potassium channels in mammalian brain. *J Neurosci* **23:**8077–8091.

Pallàs M and Camins A (2006) Molecular and biochemical features in Alzheimer's disease. *Curr Pharm Des* **12:**4389–4408.

Pan Y, Xu X, Tong X, and Wang X (2004) Messenger RNA and protein expression analysis of voltage-gated potassium channels in the brain of Abeta(25–35)-treated rats. *J Neurosci Res* **77:**94–99.

Pannaccione A, Boscia F, Scorziello A, Adornetto A, Castaldo P, Sirabella R, Tagliatatela M, Di Renzo GF, and Annunziato L (2007) Up-regulation and increased activity of K$_V$3.4 channels and their accessory subunit MinK-related peptide 2 induced by amyloid peptide are involved in apoptotic neuronal death. *Mol Pharmacol* **72:**665–673.

Plant LD, Webster NJ, Boyle JP, Ramsden M, Freir DB, Peers C, and Pearson HA (2006) Amyloid beta peptide as a physiological modulator of neuronal 'A'-type K+ current. *Neurobiol Aging* **27:**1673–1683.

Ramsden M, Plant LD, Webster NJ, Vaughan PF, Henderson Z, and Pearson HA (2001) Differential effects of unaggregated and aggregated amyloid beta protein (1–40) on K+ channel currents in primary cultures of rat cerebellar granule and cortical neurones. *J Neurochem* **79:**699–712.

Schröter KH, Ruppersberg JP, Wunder F, Rettig J, Stocker M, and Pongs O (1991) Cloning and functional expression of a TEA-sensitive A-type potassium channel from rat brain. *FEBS Lett* **278:**211–216.

Vega-Saenz de Miera E, Moreno H, Fruhling D, Kentros C, and Rudy B (1992) Cloning of ShIII (Shaw-like) cDNAs encoding a novel high-voltage-activating, TEA-sensitive, type-A K+ channel. *Proc Biol Sci* **248:**9–18.

Yu SP (2003) Regulation and critical role of potassium homeostasis in apoptosis. *Prog Neurobiol* **70:**363–386.

Address correspondence to: Dr. Geoffrey W. Abbott, Starr 463, Greenberg Division of Cardiology, Weill Medical College of Cornell University, 520 East 70th Street, New York, NY 10021. E-mail: gwa2001@med.cornell.edu