1-1-2012

Potential implications of HCN channel dysfunction after subarachnoid hemorrhage

Ananth K. Vellimana
Washington University School of Medicine in St. Louis

Journal Club

Editor's Note: These short, critical reviews of recent papers in the *Journal*, written exclusively by graduate students or postdoctoral fellows, are intended to summarize the important findings of the paper and provide additional insight and commentary. For more information on the format and purpose of the Journal Club, please see http://www.jneurosci.org/misc/ifa_features.shtml.

Potential Implications of HCN Channel Dysfunction after Subarachnoid Hemorrhage

Ananth K. Vellimana
Department of Neurological Surgery, Washington University School of Medicine, St. Louis, Missouri 63110

Review of Li et al.

Subarachnoid hemorrhage (SAH) most commonly occurs due to rupture of a cerebral aneurysm, and it is responsible for approximately a quarter of all cerebrovascular deaths. Although considerable advances have been made in the understanding of the pathophysiology of SAH and its complications, it is still associated with significant mortality (~40% at 30 d) and morbidity (~50% of survivors have long-term neurologic deficits). This high morbidity and mortality can be attributed to two distinct yet similar phenomena: early brain injury and delayed cerebral ischemia (DCI). Early brain injury occurs <72 h after SAH and is characterized by pathophysiological changes that result in global hypoperfusion, blood–brain barrier breakdown, cerebral edema, and neuronal cell death. DCI begins several days after the ictus and peaks ~7 d after SAH. DCI was initially attributed to vasospasm, a phenomenon characterized by delayed narrowing of large cerebral arteries. More recently, it has been recognized that in addition to vasospasm, several other factors, including cortical spreading depolarization (CSD), microvascular dysfunction, and microthrombosis, are important contributors to DCI and subsequent neuronal cell death (Macdonald et al., 2008).

Received April 25, 2012; revised May 23, 2012; accepted May 25, 2012.

This work was supported by an American Heart Association Postdoctoral Fellowship Award (11POST7650038). I thank my mentors, Drs. Gregory J. Zipfel and Byung Hee Han, for support and valuable discussion.

The author reports no financial conflicts of interest.

Correspondence should be addressed to Dr. Ananth K. Vellimana, Department of Neurological Surgery, Washington University School of Medicine, 660 S. Euclid Avenue, Box 8057, St. Louis, MO 63110. E-mail: vellimana@gmail.com.

DOI:10.1523/JNEUROSCI.2064-12.2012

 0270-6474/12/329117-02$15.00/0

Among the non-vasospasm contributors to DCI, much evidence from animal and human studies supports a critical role for CSDs (Dreier, 2011). In brief, CSDs are characterized by spreading waves of sustained depolarization in neurons that is initiated by a disturbance of the cellular electrochemical gradient. CSDs are normally accompanied by a hemodynamic response consisting of microvascular dilatation that aims to increase tissue perfusion and meet the energy demands of the cell. However, in damaged regions of the cerebral cortex, neuronal depolarization and blood flow become inversely coupled, leading to microvascular spasm instead of dilatation. This phenomenon exacerbates the energy crisis in neurons and ultimately results in cell death.

In an article recently published in *The Journal of Neuroscience*, Li et al. (2012) examined the contribution of hyperpolarization-activated cyclic nucleotide-gated (HCN) channels to neuronal hyperexcitability after SAH. The HCN channel family is comprised of four homologous members (HCN1–4) that differ functionally in their current activation kinetics and response to cAMP. Li et al. (2012) focused on the HCN1 subtype because CSDs are easily provoked in the neocortex and hippocampus, where HCN1 channels are abundant. They performed whole-cell clamp recordings on pyramidal neurons from CA1 in hippocampal slices to examine HCN channel activity, and they mimicked SAH by adding oxyhemoglobin to the perfusate. The authors also assessed the expression of HCN1 channel subtype in the CA1 region of tissue obtained from a rat SAH model involving endovascular perforation.

A principal finding of Li et al. (2012) was that infusion of oxyhemoglobin induced firing of pyramidal neurons in CA1, and that this neuronal hyperexcitability was mediated by oxyhemoglobin-induced inhibition of HCN channels. These findings are important for several reasons. First, this *ex vivo* model builds upon previous *in vivo* studies (Dreier et al., 2000), which demonstrated that superfusion of hemoglobin onto the cortical surface in rats induced CSDs and neuronal death in the presence of high extracellular K^+ or low glucose. The study by Li et al. (2012) is therefore an important addition to the growing body of evidence supporting neuronal hyperexcitability and CSD after SAH. Second, the finding is important because a significant proportion of long-term disability in patients who survive after SAH is related to cognitive impairment. Although the etiology of SAH-induced cognitive impairment is likely multifactorial and cognitive impairment in different domains may be observed in SAH patients (Mayer et al., 2002), altered spatial memory has been demonstrated in both humans and rat models of SAH (Mayer et al., 2002; Jeon et al., 2010). Given that the hippocampus (especially the CA1 region) plays a critical role in spatial memory, it is plausible that HCN channel dysfunction after SAH might contribute to cognitive impairment in SAH patients. This notion is supported by the findings that long-term potentiation in the hippocampal Schaffer collateral pathway is impaired after experimental SAH (Tariq et al., 2010), and that, under normal conditions, pharmacological blockade of HCN channels impairs long-term potentiation in this pathway (He et al., 2010). Nevertheless,

before embarking on studies examining the contribution of HCN channel dysfunction to cognitive impairment after SAH, it is important to recognize that the role of HCN channels in spatial memory formation is complex, with different studies providing conflicting results. A third reason why oxyhemoglobin-induced inhibition of HCN channels and consequent neuronal hyperexcitability is important is that a large proportion of patients with SAH experience seizures, either in the early period after the ictus or at later time point. Presumably, early-onset seizures are due to biochemical dysfunction of neurons, while delayed-onset seizures may result from structural abnormalities due to gliosis. However, the molecular mechanisms underlying seizure onset after SAH remain poorly understood. Given the critical role of HCN channels in the regulation of neuronal excitability in various neural networks and in the pathogenesis of epilepsy in animal models and humans (Noam et al., 2011), it is possible that inhibition of these channels after SAH contributes to epileptogenesis.

Another pair of important observations by Li et al. (2012) are that NO levels alter HCN channel activity and that oxyhemoglobin-induced inhibition of HCN channels might result from scavenging of NO. Li et al. (2012) also demonstrated reversal of oxyhemoglobin-induced inhibition of HCN activity and neuronal hyperexcitability by exogenous administration of NO. Based on available evidence (Garthwaite et al., 2006), it is likely that NO affects the function of HCN through cGMP gating of these channels. However, an increase in phosphodiesterase-5 activity and consequent decrease in cGMP occurs in cortical neurons after experimental SAH (Han et al., 2012). It is therefore possible that cGMP downregulation seen *in vivo* would further exacerbate the magnitude of HCN channel inhibition and thereby enhance the degree of neuronal excitability.

A third key finding by Li et al. (2012) is that HCN1 gene and protein expression are reduced in CA1 for at least 72 h after ictus in a rat endovascular perforation model of SAH. While this favors a role for the HCN1 subtype in the aforementioned electrophysiological observations, it does not exclude the potential contribution of other subtypes, especially HCN2, which is ubiquitously expressed in the brain and is more abundant than HCN1 (Postea and Biel, 2011). Future studies using a similar experimental design in genetically modified mice that lack one or more of these channel subtypes (e.g., *HCN1* knock-out mice) may fill this lacuna.

It is also important to understand the limitations of the work of Li et al. (2012). First, the authors used oxyhemoglobin to mimic the effect of SAH. It would be interesting to see whether similar results are obtained when the slice preparation is perfused with aCSF containing red blood cell lysate, an experimental paradigm that would more closely mimic the CSF milieu in SAH. Performing such an experiment is critical given that HCN currents (I_h) are increased with increasing extracellular K^+ (Biel et al., 2009), and a perfusate containing red blood cell lysate would contain high levels of K^+. An *in vivo* model would be even more likely to replicate events occurring during SAH. For example, one could perform electrophysiology on hippocampal slices obtained after induction of SAH in rats via endovascular perforation or other methods. Another improvement would be to perform intracellular recording *in vivo* after induction of experimental SAH. A second limitation of the study by Li et al. (2012) is that the authors examined only pyramidal neurons in the hippocampus. Although the hippocampus is important for learning and memory, a larger portion of the long-term morbidity after SAH is due to cortical infarcts and subsequent sensorimotor deficits. Because several studies have demonstrated spreading depolarizations on the cortical surface in SAH patients, a logical next step would be to examine the function of HCN channels in pyramidal neurons of the cerebral cortex and understand their role in SAH-induced neuronal hyperexcitability and CSD. Examination of cortical neurons is also important given that alteration of HCN channel activity can have different consequences depending on the brain region and the net effect of different types of ion channels present in that particular neuronal population (Postea and Biel, 2011). A third limitation is that all the electrophysiology studies by Li et al. (2012) were performed on hippocampal slices from neonatal rats. However, SAH occurs primarily in adults, with a peak incidence of 45–55 years. Since it is well recognized that neural circuits and activity are different in adults than neonates, it is essential to examine whether the findings of this study are applicable to adults.

In summary, Li et al. (2012) provide compelling evidence to suggest that dysfunction of neuronal HCN channels may occur after SAH. Future studies need to be performed to establish this in an *in vivo* model of SAH, to characterize the subtype of HCN channels and brain regions involved, and to identify the functional consequences of HCN channel dysfunction. If the aforementioned studies are realized and cross-validated in different experimental models of SAH, modulation of HCN channel function may emerge as a new therapeutic strategy to attenuate SAH-induced neurovascular dysfunction.

References

Biel M, Wahl-Schott C, Michalakis S, Zong X (2009) Hyperpolarization-activated cation channels: from genes to function. Physiol Rev 89:847–885.

Dreier JP (2011) The role of spreading depression, spreading depolarization and spreading ischemia in neurological disease. Nat Med 17:439–447.

Dreier JP, Ebert N, Priller J, Megow D, Lindauer U, Klee R, Reuter U, Imai Y, Einhäupl KM, Victorov I, Dirnagl U (2000) Products of hemolysis in the subarachnoid space inducing spreading ischemia in the cortex and focal necrosis in rats: a model for delayed ischemic neurological deficits after subarachnoid hemorrhage? J Neurosurg 93:658–666.

Garthwaite G, Bartus K, Malcolm D, Goodwin D, Kollb-Sielecka M, Dooldeniya C, Garthwaite J (2006) Signaling from blood vessels to CNS axons through nitric oxide. J Neurosci 26:7730–7740.

Han BH, Vellimana AK, Zhou ML, Milner E, Zipfel GJ (2012) Phosphodiesterase 5 inhibition attenuates cerebral vasospasm and improves functional recovery after experimental subarachnoid hemorrhage. Neurosurgery 70: 178–186; discussion 186–187.

He W, Cheng Z, Fu G, Xu X, Lu Q, Guo L (2010) ZD7288-induced suppression of long-term potentiation was attenuated by exogenous NMDA at the schaffer collateral-CA1 synapse in the rat in vivo. Eur J Pharmacol 631:10–16.

Jeon H, Ai J, Sabri M, Tariq A, Macdonald RL (2010) Learning deficits after experimental subarachnoid hemorrhage in rats. Neuroscience 169:1805–1814.

Li B, Luo C, Tang W, Chen Z, Li Q, Hu B, Lin J, Zhu G, Zhang JH, Feng H (2012) Role of HCN channels in neuronal hyperexcitability after subarachnoid hemorrhage in rats. J Neurosci 32:3164–3175.

Macdonald RL, Kassell NF, Mayer S, Ruefenacht D, Schmiedek P, Weidauer S, Frey A, Roux S, Pasqualin A (2008) Clazosentan to overcome neurological ischemia and infarction occurring after subarachnoid hemorrhage (CONSCIOUS-1): randomized, double-blind, placebo-controlled phase 2 dose-finding trial. Stroke 39:3015–3021.

Mayer SA, Kreiter KT, Copeland D, Bernardini GL, Bates JE, Peery S, Claassen J, Du YE, Connolly ES Jr (2002) Global and domain-specific cognitive impairment and outcome after subarachnoid hemorrhage. Neurology 59:1750–1758.

Noam Y, Bernard C, Baram TZ (2011) Towards an integrated view of HCN channel role in epilepsy. Curr Opin Neurobiol 21:873–879.

Postea O, Biel M (2011) Exploring HCN channels as novel drug targets. Nat Rev Drug Discov 10:903–914.

Tariq A, Ai J, Chen G, Sabri M, Jeon H, Shang X, Macdonald RL (2010) Loss of long-term potentiation in the hippocampus after experimental subarachnoid hemorrhage in rats. Neuroscience 165:418–426.