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The Adenosine Story Goes Ionic: $Ca_V2.1$-type Ca^{2+} Channels Identified as Effectors of Adenosine's Somnogenic Actions

Commentary on Deboer et al. Reduced sleep and low adenosinergic sensitivity in *Cacna1a* R192Q mutant mice. SLEEP 2013;36:127-136.

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Attenuating the actions of the neurochemical adenosine through a cup of coffee is part of the daily wake-up procedure of many amongst us. A large body of accumulating literature, coined as the "adenosine story,"[1] indicates that adenosine is a primary sleep factor and that caffeine, coffee's active ingredient, combats adenosinergic receptor activation.[2] Adenosine results from the degradation of the energy-rich molecule ATP that is consumed in the brain during electrical and synaptic activity.[2,3] Being awake and attentive is energetically costly for the brain and markedly increases adenosinergic "tone."[2,4,5] By binding to receptor subtypes A_1 and A_{2A}, adenosine exerts diverse neuromodulatory actions throughout the brain.[6] A_1 receptors are inhibitory, G_i-coupled receptors widely expressed in wake-active areas and central to adenosine's sleep promotion.[2] But many questions about how adenosine relates to sleep homeostasis remain. Which of the cellular signaling pathways engaged by A_1 receptors are responsible for sleep induction? The new study by Deboer and colleagues[7] in this issue of *SLEEP* is the first to specify the molecular basis of an ionic pathway for the somnogenic actions of A_1 receptor activation: an inhibition of $Ca_V2.1$-type Ca^{2+} channels, members of the family of voltage-gated Ca^{2+} channels. $Ca_V2.1$ channels are involved in controlling vesicular release from presynaptic terminals; therefore, attenuation of neurotransmission at $Ca_V2.1$-expressing synapses is an important pathway for sleep induction through adenosine.

Voltage-gated, Ca^{2+}-permeable ion channels are found at both presynaptic and postsynaptic sites and are key determinants of neuronal excitability and synaptic transmission. The three members of the Ca_V2 channel group, $Ca_V2.1$ - $Ca_V2.3$, are concentrated at presynaptic terminals of excitatory and inhibitory synapses, where they typically cooperate to control synaptic release. $Ca_V2.1$ channels often mediate highly reliable and temporally precise release[8] and are found throughout the brain, including in subcortical and cortical areas involved in the regulation of vigilance states.[9-11] Moreover, these channels are susceptible to inhibition by G_i-protein-coupled neurotransmitters, such as adenosinergic A_1 receptors.[12] Adenosine dampens glutamate release at several excitatory terminals in the brain,[13] including through A_1-receptor-mediated Ca_V2 channel inhibition,[14,15] thereby controlling synaptic plasticity[16,17] and protecting neurons from insult.[18] $Ca_V2.1$ channels are thus particularly favorable candidates to contribute to adenosine's promotion of sleep.

However, testing an ion channel's involvement in adenosinergic regulation of synaptic transmission in the intact brain is tricky, since, if modified, network excitability and hence release of neurotransmitter will be altered, notably that of adenosine itself. Moreover, loss-of-function of $Ca_V2.1$ channels causes major motor disorders[19] and compensatory upregulation of other voltage-gated Ca^{2+} channels.[20] Therefore, assessing sleep-wake behavior in animals with dysfunctional channels is unlikely to provide conclusive insights about adenosine's molecular targets.

Deboer et al.[7] found an elegant solution when choosing a knock-in mouse carrying the R192Q mutation in the *Cacna1a* gene encoding the pore-forming subunit of $Ca_V2.1$ channels. This amino acid substitution in the S4 transmembrane domain decreases the sensitivity of the $Ca_V2.1$ channel to G_i-proteins, while leaving intact the maximal extent of inhibition.[21] The mutation also results in a shift in the voltage dependence of Cav2.1 channels.[21] Nevertheless, this mouse permitted study of the role of a functionally responsive Ca^{2+} channel with preserved expression levels, but compromised primarily in G-protein-mediated inhibition.[22] The hypothesis to be tested by Deboer et al.[7] was clear: if $Ca_V2.1$ channels mediate some of adenosinergic actions on sleep, then these animals should show attenuated sleep behavior when adenosine concentrations are elevated, but not when they are exceedingly low or high. Indeed, under natural sleeping conditions, R192Q knock-in mice showed less NREM sleep and substantially prolonged wake periods during the dark, active phase, whereas sleep-wake behavior in the light was unaltered. Additionally, mice responded normally to sleep deprivation and showed preserved rebound sleep and elevated slow-wave activity, consistent with the idea that high adenosine levels may inhibit $Ca_V2.1$ channels close-to-maximally. Again, however, animals were more active in the ensuing dark period, during which adenosine levels are lowered. Intriguingly, when the mutated animals were injected with either caffeine or the specific A_1 receptor agonist cyclopentyladenosine, they showed a more rapid reversal to the pre-drug sleep-wake behavior. $Ca_V2.1$ channels are thus effectors acting in proportion to both increases and decreases in adenosine, indicating that they are ongoing bidirectional monitors of adenosinergic "tone" and mediate the duration of its actions on sleep-wake behavior. In agreement with this, R192Q mice live under a constantly elevated sleep pressure, as adenosine levels are no longer funneled through Cav2.1 channels to promote sleep.

In conclusion, the work presented by Deboer and colleagues[7] significantly advances understanding of the signaling pathways recruited by adenosine that are important for somnogenesis.

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One should certainly keep in mind that in the R192Q mouse, G_i-mediated channel inhibition is compromised throughout the brain, hampering many important presynaptic modulators and altering the voltage-dependence of the Cav2.1 channels. In humans, the R192Q substitution occurs from a spontaneous missense mutation causing familial hemiplegic migraine.[22,23] Nevertheless, the choice of this mouse is particularly fortuitous, since it reveals much of adenosine's actions. So far, the sleep-inducing properties of adenosine have been dominantly associated to adenosine's direct hyperpolarization of wake-promoting neurons, to the promotion of bursting activity in thalamus,[24] and to concomitant direct excitation of sleep-promoting neurons by A_{2A} receptors.[6] These postsynaptic actions are undoubtedly important, but Deboer et al.[7] now bring to the forefront that adenosine steadily suppresses an ongoing presynaptic drive, notably at terminals expressing $Ca_V2.1$ channels. How exactly in the brain weakening of this drive leads to sleep remains to be discovered, but a potential site for such inhibition is the ascending cholinergic system, a primary site of adenosine action.[4,13,25] Indeed, *in vitro* studies show that glutamate release onto basal forebrain cholinergic neurons is dominated by $Ca_V2.1$ channels[10] and, in pontine tegmentum, inhibited by adenosine.[26] Moreover, adenosine inhibits glutamatergic afferents onto hypocretin/orexin neurons via inhibition of presynaptic voltage-gated Ca^{2+} channels.[11] Conversely, A_1-mediated presynaptic effects in thalamus promote desynchronization of thalamic sleep-related network activity.[27] Pioneering work of the kind presented by Deboer et al. will help to balance the relative importance of adenosine's multiple sites of action, which is critical for understanding the mechanisms of sleep regulation, and for future endeavors in specific drug development and medical treatment for sleep disorders.

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DISCLOSURE STATEMENT

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REFERENCES

1. Strecker RE, Basheer R, McKenna JT, McCarley RW. Another chapter in the adenosine story. Sleep 2006;29:426-8.
2. Landolt HP. Sleep homeostasis: a role for adenosine in humans? Biochem Pharmacol 2008;75:2070-9.
3. Harris JJ, Jolivet R, Attwell D. Synaptic energy use and supply. Neuron 2012;75:762-77.
4. Porkka-Heiskanen T, Strecker RE, McCarley RW. Brain site-specificity of extracellular adenosine concentration changes during sleep deprivation and spontaneous sleep: an in vivo microdialysis study. Neuroscience 2000;99:507-17.
5. Schmitt LI, Sims RE, Dale N, Haydon PG. Wakefulness affects synaptic and network activity by increasing extracellular, astrocyte derived adenosine. J Neurosci 2012;32:4417-25.
6. Brown RE, Basheer R, McKenna JT, Strecker RE, McCarley RW. Control of sleep and wakefulness. Physiol Rev 2012;92:1087-187.
7. Deboer T, van Diepen HC, Ferrari MD, Van den Maagdenberg AMJM, Meijer JH. Reduced sleep and low adenosinergic sensitivity in *Cacna1a* R192Q mutant mice. Sleep 2013;36:127-36.
8. Eggermann E, Bucurenciu I, Goswami SP, Jonas P. Nanodomain coupling between Ca^{2+} channels and sensors of exocytosis at fast mammalian synapses. Nat Rev Neurosci 2012;13:7-21.
9. Westenbroek RE, Sakurai T, Elliott EM, et al. Immunochemical identification and subcellular distribution of the α_{1A} subunits of brain calcium channels. J Neurosci 1995;15:6403-18.
10. Momiyama T. Developmental increase in D_1-like dopamine receptor-mediated inhibition of glutamatergic transmission through P/Q-type channel regulation in the basal forebrain of rats. Eur J Neurosci 2010;32:579-90.
11. Liu ZW, Gao XB. Adenosine inhibits activity of hypocretin/orexin neurons via A1 receptor in the lateral hypothalamus: a possible sleep-promoting effect. J Neurophysiol 2007;97:837-48.
12. Dolphin AC. G protein modulation of voltage-gated calcium channels. Pharmacol Rev 2003;55:607-27.
13. Bjorness TE, Greene RW. Adenosine and sleep. Curr Neuropharmacol 2009;7:238-45.
14. Gundlfinger A, Bischofberger J, Johenning FW, Torvinen M, Schmitz D, Breustedt J. Adenosine modulates transmission at the hippocampal mossy fibre synapse via direct inhibition of presynaptic calcium channels. J Physiol 2007;582:263-77.
15. Yang SC, Chiu TH, Yang HW, Min MY. Presynaptic adenosine A_1 receptors modulate excitatory synaptic transmission in the posterior piriform cortex in rats. Brain Res 2007;1156:67-79.
16. Brager DH, Thompson SM. Activity-dependent release of adenosine contributes to short-term depression at CA3-CA1 synapses in rat hippocampus. J Neurophysiol 2003;89:22-6.
17. Moore KA, Nicoll RA, Schmitz D. Adenosine gates synaptic plasticity at hippocampal mossy fiber synapses. Proc Natl Acad Sci U S A 2003;100:14397-402.
18. Fredholm BB, Chen JF, Masino SA, Vaugeois, J-M. Actions of adenosine at its receptors in the CNS: insights from knockouts and drugs. Annu Rev Pharmacol Toxicol 2005;45:385-412.
19. Rajakulendran S, Schorge S, Kullmann DM, Hanna MG. Dysfunction of the $Ca_V2.1$ calcium channel in cerebellar ataxias. F1000 Biol Rep 2010;2:4.
20. Etheredge JA, Murchison D, Abbott LC, Griffith WH. Functional compensation by other voltage-gated Ca^{2+} channels in mouse basal forebrain neurons with $Ca_V2.1$ mutations. Brain Res 2007;1140:105-19.
21. van den Maagdenberg AM, Pietrobon D, Pizzorusso T, et al. A Cacna1a knockin migraine mouse model with increased susceptibility to cortical spreading depression. Neuron 2004;41:701-10.
22. Pietrobon D. Insights into migraine mechanisms and $Ca_V2.1$ calcium channel function from mouse models of familial hemiplegic migraine. J Physiol 2010;588:1871-8.
23. Uchitel OD, Inchauspe CG, Urbano FJ, Di Guilmi MN. $Ca_V2.1$ voltage activated calcium channels and synaptic transmission in familial hemiplegic migraine pathogenesis. J Physiol Paris 2012;106:12-22.
24. Pape HC. Adenosine promotes burst activity in guinea-pig geniculocortical neurones through two different ionic mechanisms. J Physiol 1992;447:729-53.
25. Bjorness TE, Kelly CL, Gao T, Poffenberger V, Greene RW. Control and function of the homeostatic sleep response by adenosine A_1 receptors. J Neurosci 2009;29:1267-76.
26. Arrigoni E, Rainnie DG, McCarley RW, Greene RW. Adenosine-mediated presynaptic modulation of glutamatergic transmission in the laterodorsal tegmentum. J Neurosci 2001;21:1076-85.
27. Ulrich D, Huguenard JR. Purinergic inhibition of GABA and glutamate release in the thalamus: implications for thalamic network activity. Neuron 1995;15:909-18.