Sodium-potassium ATPase emerges as a player in hippocampal phenotypes of Angelman syndrome mice

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Hallengren JJ, Vaden RJ. Sodium-potassium ATPase emerges as a player in hippocampal phenotypes of Angelman syndrome mice. J Neurophysiol 112: 5–8, 2014. First published February 5, 2014; doi:10.1152/jn.00760.2013.—Angelman syndrome is a neurodevelopmental disorder characterized by intellectual disabilities, ataxia, and unusually happy affect. The hippocampal pyramidal cells of Angelman syndrome model mice have altered intrinsic membrane properties, which Kaphzan et al. (Cell Rep 4: 405–412, 2013) demonstrate can be corrected by genetic reduction of the α1-subunit of the sodium-potassium ATPase. Intriguingly, this manipulation also restores hippocampal long-term potentiation and learning. In this Neuro Forum, we discuss translational implications of this work and remaining questions left in its wake.

Angelman syndrome; sodium-potassium ATPase; intrinsic excitability; long-term potentiation

ANIELMAN SYNDROME (AS) was first described in 1965 by Dr. Harry Angelman, who proposed that three unrelated children presenting with intellectual disabilities, lack of language development, easily provoked laughter, and microcephaly came by these traits through a common genetic mechanism (Angelman 1965). Although Dr. Angelman’s moniker of “happy puppet syndrome” was soon discarded, AS became a rare but regularly diagnosed neurodevelopmental disorder in the decades that followed. In the late 1990s, it was discovered that the majority of patients with AS have a missing or mutated maternal copy of Ube3a, which encodes the ubiquitin ligase E6-AP (Kishino et al. 1997). The paternal copy of Ube3a is silenced in many brain regions due to genetic imprinting (Rougeulle et al. 1997), rendering these regions devoid of E6-AP when the maternal copy contains a loss of function mutation.

Elucidation of the genetic underpinnings of AS enable the disorder to be effectively modeled in mice. The most widely studied AS model is the Ube3a<sup>m<sub>−/−</sub>p<sup>+</sup></sup> mouse, which, similar to AS patients, harbors a loss of function mutation in the maternal copy of Ube3a (Jiang et al. 1998). Ube3a<sup>m<sub>−/−</sub>p<sup>+</sup></sup> mice recapitulate many features of human AS, including motor deficits, reduced brain weight, increased seizure susceptibility, and deficits in learning and memory (Jiang et al. 1998). The learning and memory deficits in AS mice are correlated with a marked decrease in hippocampal long-term potentiation (LTP) (Jiang et al. 1998). Many investigations have focused on the causes underlying the LTP deficit, with the assumption that these same mechanisms underlie the deficits in learning and memory in AS mice and, in turn, the intellectual disabilities observed in AS patients (Jana 2012). But what other mechanisms besides reduced synaptic plasticity might contribute to AS?

In pioneering studies, Eric Klann’s group has demonstrated a role for altered intrinsic excitability in the neuropathology of AS mice. In 2011, Kaphzan et al. showed that hippocampal pyramidal cells of AS mice have lower threshold potentials with larger and faster action potentials and hyperpolarized resting membrane potentials. These alterations in both passive and active intrinsic properties persist when the membrane potential is normalized by electrical manipulation, suggesting altered ion movement through the membrane. Investigation of the abundance of axon initial segment (AIS) proteins demonstrated increases in the α1-subunit of the sodium potassium ATPase (α1-NaKA), the voltage-gated sodium channel Na<sub>v</sub>1.6, and the AIS scaffolding protein ankyrin-G (ank-G), as well as increased AIS length in hippocampal pyramidal cells. Although the role of E6-AP in these AIS alterations is unclear, the authors convincingly demonstrated that the increase in α1-NaKA precedes the changes in AIS length and composition, and that in areas of the brain in which α1-NaKA abundance is unaltered, so too is the AIS. Indeed, perturbations in both AIS composition and intrinsic membrane properties seem to be restricted to hippocampus. Based on these data, Kaphzan et al. (2011) hypothesized that increased α1-NaKA leads to hyperpolarization of the resting membrane potential (Fig. 1A) and that Na<sub>v</sub>1.6 expression and AIS length increase to restore neuronal excitability.

In a recently published follow up paper, the focus of this article, Kaphzan et al. (2013) tested this hypothesis by genetically reducing α1-NaKA in AS mice. They generated AS mice heterozygous for the deletion of α1-NaKA (Ube3a<sup>m<sub>−/−</sub>p<sup>+</sup> α1-NaKA<sup>−/+</sup></sup>), which they termed double knockout, or dKO, mice. Hippocampal α1-NaKA levels in dKO mice are ~60% of those observed in wild-type mice, and 30% of those observed in AS mice. Consistent with the hypothesis of Kaphzan et al., dKO mice have reduced expression of Na<sub>v</sub>1.6 and ank-G compared with AS mice, and neither the abundance of AIS proteins nor the length of the AIS itself differs significantly from what is observed in wild-type mice. Importantly, non-AS mice that are heterozygous for the deletion of α1-NaKA (Ube3a<sup>m<sub>−/−</sub>p<sup>+</sup> α1-NaKA<sup>−/+</sup></sup> do not have alterations in AIS length or the abundance of AIS proteins compared with wild-type mice. This argues that genetic reduction of α1-NaKA in AS mice does not simply mask alterations in AIS length and ion movement through the membrane.

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Fig. 1. Na\(^{+}/\)K\(^{+}\) exchange and signal transduction activity of the sodium-potassium ATPase. A: the sodium-potassium ATPase hydrolyzes ATP and translocates Na\(^{+}\), K\(^{+}\), and ADP into and out of the cell. Ouabain binding stimulates \(\alpha_1\)-NaKA signal transduction activity to cause the release of calcium from the endoplasmic reticulum. DAG, diacylglycerol; PIP\(_2\), phosphatidylinositol 4,5-bisphosphate.

composition by introducing off-setting changes, but, instead, modifies the mechanism by which these changes arise.

When Kaphzan et al. (2011) first reported elevation of \(\alpha_1\)-NaKA and Na\(_{\alpha_1.6}\) in AS mice, they proposed that increased \(\alpha_1\)-NaKA activity underlies the hyperpolarized resting membrane potential observed in AS hippocampal neurons, and that increased Na\(_{\alpha_1.6}\) density subserves the increase in action potential amplitude and maximum rate of rise. This leads to the prediction that reduction of \(\alpha_1\)-NaKA levels, and the consequent decrease in Na\(_{\alpha_1.6}\) density, will reverse the increases in intrinsic excitability that drove the changes in AS hippocampal pyramidal cells to a greater extent than \(\alpha_1\)-NaKA under normal conditions, and only when the abundance of \(\alpha_1\)-NaKA is increased, as in AS mice, does it significantly influence pyramidal cell intrinsic membrane properties.

Kaphzan et al. (2013) next investigated whether the restoration of intrinsic membrane properties in AS mice brought about by genetic reduction of \(\alpha_1\)-NaKA was accompanied by restoration of hippocampus-dependent learning and memory and its correlate, reduced LTP. Somewhat surprisingly, they found that deficits in hippocampal LTP, long-term contextual fear conditioning, and Morris water maze observed in AS mice are absent in dKO mice, implicating increased \(\alpha_1\)-NaKA as a central factor in the synaptic plasticity and cognitive deficits seen in AS mice. Because there is no change in hippocampal paired-pulse facilitation before and after LTP induction in dKO mice, the authors argue that the rescue of the AS LTP deficits does not occur through a presynaptic mechanism such as increased release probability, but, instead, occurs through a postsynaptic mechanism. This conclusion is consistent with the reduction of dendritic spine size and density thought to be a structural correlate of the reduced synaptic plasticity in the AS hippocampus (Condon et al. 2013).

Unfortunately, Kaphzan et al. (2013) did not investigate whether the rescue of LTP in dKO mice was accompanied by restoration of spine density or morphology, nor did they explore possible molecular mechanisms for the LTP rescue. A breadth of recent papers has identified altered postsynaptic signaling cascades in AS mice; increased inhibitory auto-phosphorylation of calcium/calmodulin-dependent kinase II (CaMKII) (Weeber et al. 2003) and reduced signaling of brain-derived neurotrophic factor (BDNF) through its receptor tropomyosin-related kinase B (TrkB) (Cao et al. 2013) are two notable examples because both have been linked to experience-dependent dendritic spine growth (Jourdain et al. 2003). As is the case with genetic reduction of \(\alpha_1\)-NaKA, correction of these alterations leads to improved LTP and/or learning (Cao et al. 2013; van Woerden et al. 2007). While this raises the question of whether genetic reduction of \(\alpha_1\)-NaKA restores LTP through one of these mechanisms, it is difficult to connect the changes that Kaphzan et al. (2013) report in intrinsic excitability to BDNF signaling, phosphorylation of CaMKII, or even to LTP in general. However, in addition to maintaining electrochemical gradients of sodium and potassium ions, the \(\alpha_1\)-subunit of the sodium-potassium pump directly interacts with the IP\(_3\) receptor and phospholipase C gamma (PLC\(_\gamma\)), which cleaves the membrane phospholipid phosphatidylinositol 4,5-bisphosphate (PIP\(_2\)) into inositol triphosphate (IP\(_3\)) and diacylglycerol (Fig. 1B) (Aperia 2007). \(\alpha_1\)-NaKA therefore both interacts with a key effector of BDNF/TrkB, PLC\(_\gamma\), and facilitates the release of calcium from endoplasmic reticulum stores, which could impact CaMKII phosphorylation, lending support to the idea that the improved LTP and learning observed in dKO mice could derive from manipulation of one of these pathways.

Regardless of whether \(\alpha_1\)-NaKA can alter BDNF signaling, the activity of CaMKII, or dendritic spine density, the value of the current work to the development of therapeutic interventions in AS rests on the question of whether reduction of
NaKA’s cation exchange activity or signal transduction activity underlies the improved cognition observed in dKO mice (Fig. 1). If the improvement is due to reduced Na⁺/K⁺ exchange, then NaKA inhibitors like digoxin, which is currently approved to treat cardiac arrhythmias, may be beneficial in AS patients. However, these drugs stimulate NaKA’s signal transduction activity (Aperia 2007), and, therefore, if dKO mice have improved learning and memory due to decreased NaKA-driven signaling, current inhibitors may exacerbate the intellectual disabilities in AS patients. Another possibility worth considering is that the overabundance of α1-NaKA in AS mice increases the amount of time during which both gates of the pump are open, allowing ions to flow into and out of the cell according to their concentration gradients (Ashcroft et al. 2009).

In addition to profound cognitive deficits, 90% of AS patients also suffer from seizures (Thibert et al. 2009). Altered GABAergic transmission has been proposed to contribute to AS epilepsy because GABRB3, which encodes the β3-subunit of the GABA_A receptor, is in the AS critical region of human chromosome 15 (Dan and Boyd 2003) and GABRB3 knockout mice phenocopy AS seizures (DeLorey et al. 1998). A stumbling block for this line of reasoning is that both AS patients with no perturbation of GABRB3 and Ube3a<sup>m<sup>+/−</sup></sup> mice have increased seizure susceptibility, arguing that loss of E6-AP can lead to epilepsy independent of GABRB3 disruption (Dan and Boyd 2003). Recently, Wallace et al. (2012) demonstrated that Ube3a<sup>m<sup>+/−</sup></sup> mice have reduced GABAergic transmission onto L3/4 pyramidal cells in visual cortex, prompting the hypothesis that excitatory/inhibitory imbalance could underlie the increased seizure susceptibility in AS mice. However, both hypoinhibition and hyperexcitation can contribute to epileptogenesis. In the current work, Kaphzan et al. (2013) demonstrate an α1-NaKA-dependent increase in Na<sub>a</sub>,1.6 at the AIS of hippocampal pyramidal cells of AS mice, consistent with proexcitatory changes observed in sodium channels in both epileptic patients (Whitaker et al. 2001) and animal models of epilepsy (Blumenfeld et al. 2009). Indeed, elevation of Na<sub>a</sub>,1.6 has been demonstrated in several epilepsy models (Blumenfeld et al. 2009; Hargus et al. 2013), suggesting an additional mechanism by which seizures may develop in AS mice. Kaphzan et al. (2013) do not report whether seizure susceptibility is rescued in dKO mice, but, given the debilitating effect of epilepsy on AS patients and their caregivers (Thibert et al. 2009), this possibility warrants further research.

Finally, the biggest open question remains how dysfunction of E6-AP, the ubiquitin ligase encoded by Ube3a, leads to increased α1-NaKA abundance in AS mice. The most parsimonious suggestion, given the role of E6-AP-mediated ubiquitination in targeting substrates to the proteasome, is that loss of E6-AP interferes with the degradation of α1-NaKA. In support of this, α1-NaKA has been reported to be ubiquitinated (Coppi and Guidotti 1997), but Kaphzan et al. (2011) demonstrated that E6-AP does not directly interact with α1-NaKA. Furthermore, there is evidence at α1-NaKA, like many large, transmembrane proteins, is degraded by the lysosome (Lecuona et al. 2009). These findings argue that α1-NaKA is not targeted for degradation by E6-AP. The abundance of the synaptic protein Arc is also increased in the absence of E6-AP (Greer et al. 2010), and a recent study suggests that this derives from stimulation of estradiol-induced Arc transcription in the absence of E6-AP (Kuhnle et al. 2013). The abundance of α1-NaKA has also been shown to be sensitive to estradiol (Gracelli et al. 2012), opening the possibility that E6-AP regulates the levels of α1-NaKA transcriptionally as opposed to posttranslationally.

In summary, the data presented by Kaphzan et al. (2013) demonstrate a role for α1-NaKA in many of the hippocampal phenotypes of AS mice. Future work should concentrate on determining the mechanism by which E6-AP dysfunction causes increased α1-NaKA abundance, if increased NaKA activity or signal transduction underlies the LTP and learning and memory deficits in AS mice, and if alterations in intrinsic excitability contribute to the increased seizure susceptibility in AS mice. Although Kaphzan et al. do not comment on whether motor dysfunction, a canonical feature of AS, is rescued in dKO mice, this could prove another profitable line of investigation. Recent evidence suggests that reduced inhibition in the cerebellum underlies AS motor deficits (Egawa et al. 2012), and that NaKA inhibition has been demonstrated to increase tonic GABA currents onto cerebellar granule cells (Diaz et al. 2013). Beyond AS, this work complements previous reports of NaKA abnormalities in human patients with autism (Al-Mosalem et al. 2009), which shares an overlapping spectra of cognitive deficits with AS, as well as increased levels of the NaKA modulator FXYD1 in Rett syndrome (RS) model mice and RS patients (Deng et al. 2007). Increased FXYD1 causes NaKA hypofunction, and deletion of the FXYD1 gene results in improvement of the cognitive symptoms of RS mice (Matagane et al. 2013). Taken together, these findings point to an unexpected role for NaKA in cognition and intellectual disabilities.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

J.J.H. and R.J.V. prepared figures; J.J.H. drafted manuscript; J.J.H. and R.J.V. edited and revised manuscript; J.J.H. and R.J.V. approved final version of manuscript.

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