Epilepsy Currents
2020, Vol. 20(5) 309-311

DOI: 10.1177/1535759720948440
journals.sagepub.com/home/epi
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How Many Angels Can Dance on the Head of a Patch Pipette? Understanding Neuronal Hyperexcitability in Angelman Syndrome

Potassium Channel Dysfunction in Human Neuronal Models of Angelman Syndrome

Sun AX, Yuan Q, Fukuda M, Yu W, Yan H, Lim GGY, Nai MH, D'Agostino GA, Tran H-D, Itahana Y, Wang D, Lokman H, Itahana K, Lim SWL, Tang J, Chang YY, Zhang M, Cook SA, Rackham OJL, Lim CT, Tan EK, Ng HH, Lim KL, Jiang Y-H, Je HS. *Science*. 2019;366(6472):1486-1492. doi: 10.1126/science.aav5386

Disruptions in the ubiquitin protein ligase E3A (*UBE3A*) gene cause Angelman syndrome (AS). Whereas AS model mice have associated synaptic dysfunction and altered plasticity with abnormal behavior, whether similar or other mechanisms contribute to network hyperactivity and epilepsy susceptibility in AS patients remains unclear. Using human neurons and brain organoids, we demonstrate that UBE3A suppresses neuronal hyperexcitability via ubiquitin-mediated degradation of calcium and voltage-dependent big potassium (BK) channels. We provide evidence that augmented BK channel activity manifests as increased intrinsic excitability in individual neurons and subsequent network synchronization. Big potassium antagonists normalized neuronal excitability in both human and mouse neurons and ameliorated seizure susceptibility in an AS mouse model. Our findings suggest that BK channelopathy underlies epilepsy in AS and support the use of human cells to model human developmental diseases.

Commentary

Angelman syndrome is a rare, fascinating neurodevelopmental disorder, about which our understanding has come full circle—from astute clinical observations of a country physician to a genetic understanding of etiology and to potential treatments based on sites of pathophysiological dysfunction. In 1965, Dr Harry Angelman, a physician in Lancashire, England, reported 3 unrelated children with a similar clinical phenotype. Each child had dysmorphic facies (angular features, wide mouth, protruding tongue, prognathia, microbrachycephaly), a happy disposition with paroxysmal bouts of laughter, puppet-like jerky/ataxic movements, hypotonia, intellectual disability, and seizures. Dr Angelman stated that the children had a "superficial resemblance to puppets, an unscientific name but one which may provide for easy identification."[1] Thus, the "happy puppet syndrome" was born, now known by the eponym, Angelman syndrome. Over the next several decades, the genetics and molecular basis of Angelman syndrome emerged. It is now known that more than 90% of Angelman syndrome is caused by a loss-of-function mutation of the *UBE3A* gene that encodes the protein E3 ubiquitin ligase, located on chromosome 15q11-13 (an imprinted region, with the clinical phenotype dependent on whether the mutation is inherited from the mother or father). E3 ubiquitin ligase protein, maternally expressed in all neurons, tags proteins for intracellular degradation. Therefore, UBE3A mutation results in accumulation and dysfunction of proteins ordinarily destined for ubiquination and degradation by E3 ubiquitin ligase.[2,3]

More than 90% of affected individuals have epilepsy, usually starting in early childhood. A variety of seizure types is observed, including myoclonic, atypical absence, atonic, and generalized tonic-clonic; focal seizures can also occur. The seizures in Angelman syndrome are typically refractory, underscoring the need for better therapies, as uncontrolled seizures might exacerbate the already impaired cognitive dysfunction in these patients. Despite repeated demonstrations of cortical hyperexcitability in Angelman syndrome, the underlying mechanisms are not yet fully explained.

Mouse models, primarily involving *Ube3a* gene deletion, mimic many of the clinical and molecular aspects of Angelman syndrome. These mice have developmental delays, ataxic gaits, seizures, synaptic dysfunction, and altered cortical plasticity. In both humans and Angelman syndrome mice, seizures are often very difficult to control, suggesting a severe alteration of the neuronal inhibition/excitation balance.[4] In that regard, genes for some GABA receptor subunits are found in the deleted region, but many affected individuals do not harbor a mutation of gamma-aminobutyric acid (GABA) receptors.[5] Overall, mouse models have not afforded a satisfactory explanation for seizures.

In this context, the current article evaluates the role of potassium channels in the excitability of neurons in Angelman syndrome.[6] The technical advances utilized by the authors are noteworthy. First, they use human cells, which adds an important dimension of clinical relevance. Second, the authors expanded their observations to 3-dimensional brain organoids (derived from neurons with UBE3A knocked out), and even extended their results to an existing mouse model of Angelman syndrome. With this battery of approaches, the authors pursued a series of elegant experiments to show that neuronal hyperexcitability in Angelman syndrome could be due to a channelopathy of BK channels, which are calcium-mediated large-conductance potassium channels (B for big, K for potassium) that underlie the fast afterhyperpolarization (fAHP) and contribute to the regulation of neuronal excitability and repetitive action potential firing.[7]

First, the authors used CRISPR-Cas9 technology to knock out UBE3A from a human embryonic stem cell (hESC) line. When neuronal differentiation was induced, electrically mature neurons resulted, allowing the authors to compare wild type (WT) and UBE3A knockout cells. Since both cell types looked normal structurally, the authors investigated electrophysiological properties using whole cell patch clamping, finding that cells from knockouts fired significantly more action potentials in response to a given current pulse than WT cells. Voltage-dependent sodium and potassium channels were similar in both cell lines, but fAHP amplitudes were markedly augmented in knockout cells. When UBE3A was restored in the knockouts, normal action potential firing and fAHP amplitudes resulted. These experiments demonstrated that loss of UBE3A results in enhanced neuronal excitability, with augmented fAHPs.

Next, the authors investigated whether increased BK protein levels contributed to the larger currents recorded in knockout cells, using atomic force microscopy to evaluate densities of BK protein at different locations along the cell body. Indeed, increased BK protein levels were found in the knockouts. Importantly, BK channel antagonists (eg, paxilline) decreased the firing rate of these cells and restored fAHP amplitudes. The authors also determined that the BK protein was a substrate for posttranslational UBE3A ubiquitination and proteosomal degradation.

While UBE3A-deficient cultured stem cells provided a plausible mechanism for hyperexcitability, complex network firing cannot be studied using such a system. Therefore, the authors used 3-dimensional organoids ("mini-brains") derived from human pluripotent stem cells with UBE3A knocked out. The mutant and WT organoids had very similar cell types, composition, and lamination. Similar to the results in hESC lines, the authors found that in UBE3A knockout organoids, excitability was increased with larger fAHPs, higher BK protein levels, and restoration of normal excitability by BK antagonists. Using 2-photon calcium imaging to study network synchronization, they found that while WT neurons fired in a random, stochastic manner, those from UBE3A knockouts fired synchronously, often in bursts, thus demonstrating enhanced hyperexcitability. Paxilline likewise restored the firing in knockouts to a pattern similar to WT.

Finally, the authors took their model full-circle and evaluated whether similar pathophysiological changes occurred in a mouse model of Angelman syndrome, involving maternal deletion of Ube3a with resultant epilepsy, learning defects, and motor dysfunction.[8,9] Using several different seizure induction methods, they found that the mice without Ube3a had enhanced susceptibility to flurothyl- and picrotoxin-induced seizures. The increased seizure susceptibility was reversed with BK antagonists. Electroencephalograms (EEGs) in freely-moving mice demonstrated increased spectral changes in total power and increased delta range rhythms, as shown previously[10]; again, BK antagonists normalized the EEG changes.

At this point, the astute reader might wonder why a larger AHP could account for *increased* excitability and seizures. Typically, outward current through potassium currents during the AHP hyperpolarizes and stabilizes the membrane potential. But here, the data suggest that UBE3A mutation results in less degradation of the BK protein, enhanced BK channel function, and a larger fAHP. This apparent paradox is resolved when considering that BK channels are activated by both voltage (depolarization) and increased intracellular calcium, that is, they are "bidirectional" or dual-action in nature. The fAHP can mediate either excitation or inhibition, depending on the exact membrane potential and how rapidly the BK conductance is activating or recovering.[11] Therefore, it is not contradictory to find that increased BK function can actually be hyperexcitatory. In support of that idea, both loss- and gain-of-function human mutations of BK channel function have been described.[12]

Overall, this is a remarkable paper that rigorously evaluates the mechanism of increased seizure susceptibility in Angelman syndrome. Using multiple approaches, the authors demonstrate convincingly that the BK channel underlying the fAHP is markedly enhanced in neurons with UBE3A underexpression. Therefore, the BK protein is likely to be increased in neurons of individuals with Angelman syndrome, pointing to an unexpected potential avenue for therapy. While it is uncertain whether this mechanism is the primary or only pathophysiological defect in Angelman syndrome, the results indicate that the sites of possible dysfunction might well involve one or more channelopathies. Logically, future studies could focus on potential clinical BK antagonists,[13,14] and evaluate other forms of AHP following repetitive firing (ie, those with slow- or medium-range kinetics). Dr Angelman would undoubtedly be pleased to see that his initial clinical observations have not only yielded an understanding of the molecular and genetic aspects of the syndrome that bears his name, but now additionally may provide mechanism-specific novel treatment for one of the most disabling features of the syndrome—intractable seizures.

By Carl E. Stafstrom

ORCID iD

Carl E. Stafstrom https://orcid.org/0000-0002-4432-2453

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