Shedding the Epilepsy Comorbidity in Alzheimer’s Disease

Postnatal Disruption of the Disintegrin/Metalloproteinase ADAM10 in Brain Causes Epileptic Seizures, Learning Deficits, Altered Spine Morphology, and Defective Synaptic Functions.


The metalloproteinase ADAM10 is of importance for Notch-dependent cortical brain development. The protease is tightly linked with α-secretase activity toward the amyloid precursor protein (APP) substrate. Increasing ADAM10 activity is suggested as a therapy to prevent the production of the neurotoxic amyloid β (Aβ) peptide in Alzheimer’s disease. To investigate the function of ADAM10 in postnatal brain, we generated Adam10 conditional knock-out (A10cKO) mice using a CamKIIα-Cre deleter strain. The lack of ADAM10 protein expression was evident in the brain cortex leading to a reduced generation of Aβ-peptide and increased levels of sAPPβ and endogenous Aβ peptides. The A10cKO mice are characterized by weight loss and increased mortality after weaning associated with seizures. Behavioral comparison of adult mice revealed that the loss of ADAM10 in the A10cKO mice resulted in decreased neuromotor abilities and reduced learning performance, which were associated with altered vivo network activities in the hippocampal CA1 region and impaired synaptic function. Histological and ultrastructural analysis of ADAM10-depleted brain revealed astrogliosis, microglia activation, and impaired number and altered morphology of postsynaptic spine structures. A defect in spine morphology was further supported by a reduction of the expression of NMDA receptors subunit 2A and 2B. The reduced shedding of essential postsynaptic cell adhesion proteins such as N-Cadherin, Nectin-1, and APP may explain the postsynaptic defects and the impaired learning, altered network activity, and synaptic plasticity of the A10cKO mice. Our study reveals that ADAM10 is instrumental for synaptic and neuronal network function in the adult murine brain.

Commentary

Epilepsy is a comorbidity in patients with Alzheimer’s disease (AD). The cleavage of amyloid precursor protein (APP) by β- and γ-secretases produces amyloid β peptides, which are the main component of amyloid plaques. Alternatively, APP may be cleaved by an α-secretase to form the soluble fragment sAPPα, which promotes neuronal survival. Shifting the proteolysis of APP from the amyloidogenic pathway to α-secretase generation is a potential therapeutic strategy for AD and associated epilepsy.

Molecules that remove extracellular domains from transmembrane proteins are collectively known as “sheddases,” and the largest family is the ADAM (a disintegrin and metalloprotease) family. Based on expression patterns and transgenic mouse studies, ADAM10 protein (also known as CD156c) has been proposed to be the main α-secretase in the generation of sAPPα. In overexpression studies, ADAM10 is neuroprotective in mice that overexpress APP in the kainate seizure model (1) and reduces β-amyloid deposition and cognitive dysfunction. However, total loss of the Adam10 gene in the mouse is embryonic lethal (2), and conditional deletions targeted to forebrain neuroprogenitors severely disrupted brain development, terminating in late embryonic mortality and intracranial hemorrhages (3). Thus, the role of ADAM10 as the α-secretase in the processing of APP in adult animal models could not be ascertained.

The postnatal function of ADAM10 is revealed by using a neuronal specific Cre-loxP strategy with the Adam10 floxed mouse bred with the CamKIIα-Cre driver mouse strain (4). The expression of CamKIIα commences at postnatal day 5 (P5), circumventing the embryonic and perinatal lethality of the previous mouse models. Based on CamKIIα expression patterns, ADAM10 was expected to be eliminated from the cerebral cortex, hippocampus, olfactory bulb, and amygdala, and to a lesser extent in the striatum, thalamus, and hypothalamus. Many of the Adam10/CamKIIα conditional mutant mice died around P18–P27, during the time of synaptogenesis, while some mice lived to 2 months, but they were smaller in size and weight than their control littermates. Repetitive behavioral seizures were observed as early as P14, with some cases being tonic seizures that ended with respiratory distress and death. Depth electrodes implanted into the hippocampus recorded electrographic seizures in 20 percent of the Adam10/CamKIIα conditional mutant mice and in none of the control mice. In summary, genetic deletion of Adam10 retarded growth and initiated a seizure phenotype.

During normal ontogeny, ADAM10 levels increase with postnatal age, in particular throughout synaptogenesis. The levels of ADAM10 in the targeted genetic mutant mice were measured by Western blot analysis. Decreased levels of
ADAM10 were observed as early as P5, at the beginning of the CamKIIα-mediated deletion, and continued to barely detectable levels at P17. The sAPPα levels were nearly absent in targeted regions, confirming the role of ADAM10 as the main α-secretase for APP. The balance of the soluble APP fragments was shifted to the Aβ species, which may ultimately lead to increased amyloid deposition and plaques in very young mice. In addition, the lack of sAPPα in its role in promoting neuronal survival may account for the seizure phenotype in juvenile animals. Dysregulation of APP processing is also observed in patients with Down syndrome, some of whom experience seizures during childhood. The DS phenotype is not really similar. Most childhood DS is infantile spasms and otherwise epilepsy tends to start in adulthood.

At P20, the hippocampus displayed no obvious abnormalities or neuronal loss. In older mice (20 and 30 weeks old), gliosis and activation of microglial were observed. Behavioral testing showed normal circadian cycles and open field motor activity, but decreased motor performance was reported for the rotarod test. The major deficits were observed during spatial memory testing in the Morris water maze, when the Adam10/CamKIIα conditional mutant mice were not able to learn the task during the acquisition phase and did not show preference for the target area during the memory probe test, reflecting the spatial memory deficits observed in other mouse models of AD.

The circuitry of the hippocampus was analyzed on multiple levels. Recordings of the Schaffer collateral pathway in hippocampal slices revealed no differences in basal synaptic transmission as measured by input–output curves. A significant difference in paired-pulse ratios (a measure of short-term plasticity) was noted at one of the six interstimulus intervals that were tested. However, long-term potentiation (LTP), a major mechanism for long-term memory formation, was severely attenuated in the Adam10/CamKIIα conditional mutant mice. While control mice exhibited LTP for more than 2 h, the Adam10/CamKIIα conditional mutant mice only displayed short-term potentiation for only a few minutes. The LTP deficit can be partially rescued with the addition of sAPPα to the recording solution, supporting the role of Adam10 as critical for the generation of sAPPα for normal function. In vivo hippocampal recordings under urethane anesthesia were divided into sleep-like (rapid eye movement [REM]) and non-REM sleep-like episodes. The numbers of ripples and sharp waves observed in non-REM sleep-like episodes were similar between control and Adam10/CamKIIα mutant mice. Analysis of the local field potentials (LFPs) in the REM episodes indicated reduced power spectra in the theta and gamma frequency ranges and a lower cross-frequency coupling between theta and gamma oscillations in the Adam10/CamKIIα mutant mice.

The altered electrophysiological function of the Adam10/CamKIIα mice suggested postsynaptic defects. Combined biochemical and immunohistochemical analysis demonstrated decreased levels of PSD-95 (post-synaptic density—95) scaffold molecule, and NMDA (N-methyl-D-aspartate) receptor subunits 2A, 2B, and NR1. Anatomically, the Adam10/CamKIIα conditional mutant mice had a reduction in the apical dendrites in the stratum radiatum and altered distribution of shapes of the spines. The Adam10/CamKIIα conditional mutant mice had more stubby and enlarged spines compared to the controls’ small and round spines, similar to morphologies reported in aged APP null mice and APP-deficient neurons. However, electron micrographs indicated that the synapses were regularly formed. The changes in spine morphology may be correlated with abnormal cleavage and shedding of cell adhesion molecules that serve as substrates for ADAM10, and Western blots confirmed decreased C-terminal fragments of N-cadherin and nectin-1, but normal full length N-cadherin and nectin-1 supporting loss of α-secretase activity.

The recent data support an independent role for ADAM10 in AD and epilepsy. Impaired processing of APP in the absence of Adam10 or γ-secretase may lead to increased network hyperexcitability (4, 5). Recent reports have identified two rare human mutations in the human gene Adam10, that correspond with late-onset AD (6). A promoter haplotype that increases Adam10 expression was associated with lower plaque load and higher cognitive scores in patients without AD, implying that greater Adam10 expression produced more sAPPα that served to protect the hippocampus from degeneration (7). The combination of human and animal research supports the potential for therapeutic agents that modulate Adam10 to prevent or delay AD and the epilepsy comorbidity.

by Elizabeth M. Powell, PhD

References

Instructions
The purpose of this form is to provide readers of your manuscript with information about your other interests that could influence how they receive and understand your work. Each author should submit a separate form and is responsible for the accuracy and completeness of the submitted information. The form is in four parts.

1. **Identifying information.**
   Enter your full name. If you are NOT the main contributing author, please check the box “no” and enter the name of the main contributing author in the space that appears. Provide the requested manuscript information.

2. **The work under consideration for publication.**
   This section asks for information about the work that you have submitted for publication. The time frame for this reporting is that of the work itself, from the initial conception and planning to the present. The requested information is about resources that you received, either directly or indirectly (via your institution), to enable you to complete the work. Checking “No” means that you did the work without receiving any financial support from any third party – that is, the work was supported by funds from the same institution that pays your salary and that institution did not receive third-party funds with which to pay you. If you or your institution received funds from a third party to support the work, such as a government granting agency, charitable foundation or commercial sponsor, check “Yes”. Then complete the appropriate boxes to indicate the type of support and whether the payment went to you, or to your institution, or both.

3. **Relevant financial activities outside the submitted work.**
   This section asks about your financial relationships with entities in the bio-medical arena that could be perceived to influence, or that give the appearance of potentially influencing, what you wrote in the submitted work. For example, if your article is about testing an epidermal growth factor receptor (EGFR) antagonist in lung cancer, you should report all associations with entities pursuing diagnostic or therapeutic strategies in cancer in general, not just in the area of EGFR or lung cancer.

   Report all sources of revenue paid (or promised to be paid) directly to you or your institution on your behalf over the 36 months prior to submission of the work. This should include all monies from sources with relevance to the submitted work, not just monies from the entity that sponsored the research. Please note that your interactions with the work’s sponsor that are outside the submitted work should also be listed here. If there is any question, it is usually better to disclose a relationship than not to do so.

   For grants you have received for work outside the submitted work, you should disclose support ONLY from entities that could be perceived to be affected financially by the published work, such as drug companies, or foundations supported by entities that could be perceived to have a financial stake in the outcome. Public funding sources, such as government agencies, charitable foundations or academic institutions, need not be disclosed. For example, if a government agency sponsored a study in which you have been involved and drugs were provided by a pharmaceutical company, you need only list the pharmaceutical company.

4. **Other relationships**
   Use this section to report other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work.
American Epilepsy Society

Epilepsy Currents Journal
Disclosure of Potential Conflicts of Interest

Section #1 Identifying Information

1. Today’s Date: June 19, 2014

2. First Name Elizabeth Last Name Powell Degree PhD

3. Are you the Main Assigned Author? ☑ Yes ☐ No

If no, enter your name as co-author:

4. Manuscript/Article Title: Shedding the epilepsy co-morbidity in Alzheimer’s disease

5. Journal Issue you are submitting for: Epilepsy Currents

Section #2 The Work Under Consideration for Publication

Did you or your institution at any time receive payment or services from a third party for any aspect of the submitted work (including but not limited to grants, data monitoring board, study design, manuscript preparation, statistical analysis, etc.)?

Complete each row by checking “No” or providing the requested information. If you have more than one relationship just add rows to this table.

<table>
<thead>
<tr>
<th>Type</th>
<th>No</th>
<th>Money Paid to You</th>
<th>Money to Your Institution*</th>
<th>Name of Entity</th>
<th>Comments**</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Grant</td>
<td>☑</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Consulting fee or honorarium</td>
<td>☑</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Support for travel to meetings for the study or other purposes</td>
<td>☑</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Fees for participating in review activities such as data monitoring boards, statistical analysis, end point committees, and the like</td>
<td>☑</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Payment for writing or reviewing the manuscript</td>
<td>☑</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Provision of writing assistance, medicines, equipment, or administrative support.</td>
<td>☑</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Other</td>
<td>☑</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* This means money that your institution received for your efforts on this study.

** Use this section to provide any needed explanation.
Section #3 Relevant financial activities outside the submitted work.
Place a check in the appropriate boxes in the table to indicate whether you have financial relationships (regardless of amount of compensation) with entities as described in the instructions. Use one line for each entity; add as many lines as you need by clicking the “Add” box. You should report relationships that were present during the 36 months prior to submission.

Complete each row by checking “No” or providing the requested information. If you have more than one relationship just add rows to this table.

<table>
<thead>
<tr>
<th>Type of relationship (in alphabetical order)</th>
<th>No</th>
<th>Money Paid to You</th>
<th>Money to Your Institution*</th>
<th>Name of Entity</th>
<th>Comments**</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Board membership</td>
<td>☑️</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Consultancy</td>
<td>☐️</td>
<td>X</td>
<td>NIH, Autism Speaks, Elsevier</td>
<td>Grant reviews Neuroscience, section editor</td>
<td></td>
</tr>
<tr>
<td>3. Employment</td>
<td>☐️</td>
<td>X</td>
<td>University of Maryland</td>
<td>employer</td>
<td></td>
</tr>
<tr>
<td>4. Expert testimony</td>
<td>☑️</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Grants/grants pending</td>
<td>☑️</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Payment for lectures including service on speakers bureaus</td>
<td>☑️</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Payment for manuscript preparation.</td>
<td>☑️</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. Patents (planned, pending or issued)</td>
<td>☑️</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. Royalties</td>
<td>☑️</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10. Payment for development of educational presentations</td>
<td>☑️</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11. Stock/stock options</td>
<td>☑️</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12. Travel/accommodations/meeting expenses unrelated to activities listed.**</td>
<td>☑️</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13. Other (err on the side of full disclosure)</td>
<td>☑️</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* This means money that your institution received for your efforts.
** For example, if you report a consultancy above there is no need to report travel related to that consultancy on this line.

Section #4 Other relationships
Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?

☑️ No other relationships/conditions/circumstances that present a potential conflict of interest.
☐ Yes, the following relationships/conditions/circumstances are present:
Thank you for your assistance.
Epilepsy Currents Editorial Board