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Double agent mTOR

Remodeled Cortical Inhibition Prevents Motor Seizures in Generalized Epilepsy

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Objective: Deletions of CACNA1A, encoding the α1 subunit of CaV 2.1 channels, cause epilepsy with ataxia in humans. Whereas the deletion of Cacna1a in γ-aminobutyric acidergic (GABAergic) interneurons (INs) derived from the medial ganglionic eminence (MGE) impairs cortical inhibition and causes generalized seizures in Nkx2.1Cre;Cacna1ac/c mice, the targeted deletion of Cacna1a in somatostatin-expressing INs (SOM-INs), a subset of MGE-derived INs, does not result in seizures, indicating a crucial role of parvalbumin-expressing (PV) INs. Here, we identify the cellular and network consequences of Cacna1a deletion specifically in PV-INs. Methods: We generated PVCre;Cacna1ac/c mutant mice carrying a conditional Cacna1a deletion in PV neurons and evaluated the cortical cellular and network outcomes of this mutation by combining immunohistochemical assays, in vitro electrophysiology, 2-photon imaging, and in vivo video-electroencephalographic recordings. Results: PVCre;Cacna1ac/c mice display reduced cortical perisomatic inhibition and frequent absences, but only rare motor seizures. Compared to Nkx2.1Cre;Cacna1ac/c mice, PVCre;Cacna1ac/c mice have a net increase in cortical inhibition, with a gain of dendritic inhibition through sprouting of SOM-IN axons, largely preventing motor seizures. This beneficial compensatory remodeling of cortical GABAergic innervation is mechanistic target of rapamycin complex 1 (mTORC1)-dependent, and its inhibition with rapamycin leads to a striking increase in motor seizures. Furthermore, we show that a direct chemogenic activation of cortical SOM-INs prevents motor seizures in a model of kainate-induced seizures. Interpretation: Our findings provide novel evidence suggesting that the remodeling of cortical inhibition, with an mTOR-dependent gain of dendritic inhibition, determines the seizure phenotype in generalized epilepsy and that mTOR inhibition can be detrimental in epilepsies not primarily due to mTOR hyperactivation.

Commentary

The mechanistic target of rapamycin (mTOR) pathway regulates neuronal plasticity, increases cell metabolism, and promotes neuronal growth. Mutations that increase mTOR signaling can cause tumor formation, but are also associated with a range of neurological disorders including autism, cortical dysplasia, and epilepsy. Increased mTOR pathway activation has also been observed in tissue collected from patients with temporal lobe epilepsy, but without identified mTOR pathway mutations,[1] consistent with animal research indicating that mTOR signaling is enhanced in acquired epilepsy.[2] Research in the mTOR field was originally driven by the chance discovery of the bacterial metabolite rapamycin in a soil sample from Easter Island, located in the South Pacific Ocean. Rapamycin is a powerful inhibitor of the mTOR pathway and has served as a useful pharmacologic tool. Clinical trials with rapamycin analogues have achieved promising results in controlling seizures and central nervous system tumor formation in tuberous sclerosis complex, a disease caused by inactivating mutations in the mTOR pathway suppressors TSC1 and TSC2.[3] Preclinical studies in animal models of acquired epilepsy—predicated on the observation that epileptogenic brain insults increase mTOR pathway activation—have also achieved promising results, often producing dramatic reductions in severe frequency. Intriguingly, however, a number of well-designed studies found no effect of rapamycin in several common seizure models.[4]

Work by Jiang and colleagues has unexpectedly led to a potential explanation for these discrepant effects of mTOR antagonism. They examined epilepsy-causing mutations in the gene encoding the α1 subunit of voltage-dependent calcium channels CaV2.1. Prior work from the group demonstrated that CaV2.1 loss from interneurons (INs) was sufficient to reproduce an epileptic phenotype in mice.[5] In the present study, they sought to identify which specific IN populations were critical. Mutations affecting both parvalbumin (PV)-expressing and

somatostatin (SOM)-expressing γ-aminobutyric acidergic INs led to epilepsy in the animals, while mutations affecting just PV-expressing INs produced a milder epilepsy phenotype. Mutations affecting just SOM-expressing INs didn't produce seizures at all.[5] Loss of CaV2.1 from PV INs impaired their synaptic efficiency, leading to a net reduction in inhibitory control of their targets: excitatory pyramidal cells. It makes sense, therefore, that loss of CaV2.1 from PV INs would be proconvulsant. Somatostatin-expressing INs, on the other hand, primarily target other INs, so it also makes sense that mutations targeted to just this population would not produce seizures. More curious, however, is why targeting the mutation to both PV- or SOM-expressing INs would produce a more severe epilepsy that targeting PV alone.

To begin to resolve this paradox, the group conducted electrophysiological studies in PV-targeted CaV2.1 mutants. These studies revealed that while synaptic efficiency at PV >> pyramidal cell synapses was reduced in PV-targeted mutants, overall inhibitory input to pyramidal cells was *increased*. Through a combination of elegant electrophysiological and anatomical work, the investigators discovered that in PV-targeted mutants, unaffected SOM-expressing INs undergo sprouting, providing compensatory inhibitory input to pyramidal cells. In animals in which both PV- and SOM-expressing INs are mutated, compensatory changes among the latter neurons are presumably blocked. Having observed that SOM neurons sprout in PV-targeted mutants, the investigators queried whether this growth was mediated by mTOR. Rapamycin treatment of PV-targeted mutants prevented SOM-expressing IN sprouting and greatly exacerbated the seizure phenotype in the animals. Rather than being antiepileptogenic in this model of epilepsy, mTOR inhibition blocked an anticonvulsant sprouting response.

The study by Jiang and colleagues highlights a key challenge for the epilepsy field, in which proconvulsant manipulations can be flipped to anticonvulsant manipulations based on the cellular and network properties of the affected neuronal populations. While this now appears clear in the CaV2.1 model, it may well be true for other models. The pilocarpine model of epilepsy—which has been used for decades in epilepsy research—provides a good example. Somatostatin neurons exhibit robust sprouting in pilocarpine-treated mice,[6] and rapamycin treatment of the animals is ineffective at controlling seizures.[7] Although a causal relationship has yet to be confirmed, rapamycin treatment blocks SOM neuron sprouting in this model,[8] suggesting that the treatment may be preventing a similar compensatory change to that observed by Jiang and colleagues, and therefore, obscuring any positive effects of the drug. Sprouting of SOM neurons has been described in other animal models of epilepsy and in humans with the disease,[9,10] suggesting that this is a common phenomenon. Notably, in children with tuberous sclerosis treated with the mTOR antagonist everolimus, seizure frequency was reduced in 13 of 20 patients, but increased in 3 patients.[3] Blockade of compensatory neuronal growth could contribute to the negative outcomes.

Taken together, the data suggest that mTOR signaling may mediate both proepileptogenic changes and compensatory inhibitory changes. If true, the utility of mTOR antagonism as a therapeutic strategy for epilepsy could be limited to a smaller number of epileptic conditions in which mTOR-mediated pathologic changes are dominant. Alternatively, it may be possible to further refine the use of these drugs to enhance their utility. Jiang and colleagues observed that SOM neuron sprouting was absent in 3-week-old mice and was blocked by rapamycin treatment given between weeks 3 to 6. Although additional studies are needed, if mTOR-mediated proepileptogenic effects are temporally dissociated from mTOR-mediated compensatory changes, it may be possible to develop a timed treatment regimen that could still be effective in epilepsy conditions in which mTOR plays both sides. Inhibition of mTOR signaling among only select neuronal populations—possibly using viral delivery strategies—might also be effective. Targeting only excitatory neurons for mTOR inhibition, for example, would allow compensatory changes among inhibitory neurons to proceed.

The work by Jiang and colleagues provides several take-home messages for thinking about epileptogenesis. Firstly, it demonstrates the key importance of cellular specificity. Depending on the cell population targeted, the same mutation or therapy can produce both pro- and antiepileptogenic effects. Secondly, the temporal dynamics of epileptogenesis is critical, with agents likely producing different effects when applied during different disease stages. Finally, the study clearly demonstrates a phenomenon that has long been suspected in the epilepsy field: that proepileptogenic changes can occur concurrently with anticonvulsant compensatory changes. Optimal therapeutic strategies for epilepsy, therefore, will require an understanding of both processes and the development of strategies to limit the former, while facilitating the latter.

By Steve C. Danzer

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