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Mechanisms of Subiculum Hyperexcitability in Temporal Lobe Epilepsy

Impaired KCC2 Function Triggers Interictal-Like Activity Driven by Parvalbumin-Expressing Interneurons in the Isolated Subiculum In Vitro

Anstötz M, Fiske MP, Maccaferri G. *Cereb Cortex*. 2021;31:4681-4698. doi:10.1093/cercor/bhab115

The decreased expression of the KCC2 membrane transporter in subicular neurons has been proposed to be a key epileptogenic event in temporal lobe epilepsy (TLE). Here, we have addressed this question in a reduced model in vitro and have studied the properties and mechanistic involvement of a major class of interneurons, that is, parvalbumin-expressing cells (PVs). When exposed to the KCC2 blocker VU0463271, mouse subicular slices generated hypersynchronous discharges that could be recorded electrophysiologically and visualized as clusters of co-active neurons with calcium imaging. The pharmacological profile of these events resembled interictal-like discharges in human epileptic tissue because of their dependence on GABAA and AMPA receptors. On average, PVs fired before pyramidal cells (PCs), and the area of co-active clusters was comparable to the individual axonal spread of PVs, suggesting their mechanistic involvement. Optogenetic experiments confirmed this hypothesis, as the flash-stimulation of PVs in the presence of VU0463271 initiated interictal-like discharges, whereas their optogenetic silencing suppressed network hyperexcitability. We conclude that reduced KCC2 activity in subicular networks in vitro is sufficient to induce interictal-like activity via altered GABAergic signaling from PVs without other epilepsy-related changes. This conclusion supports an epileptogenic role for impaired subicular KCC2 function during the progression of TLE.

Commentary

Increased excitability of the subiculum is a common feature of temporal lobe epilepsy (TLE). Indeed, hyperexcitable subicular neurons are seen in people with TLE and also in animal models of TLE.[1,2] As with many forms of epilepsy, impaired inhibition is thought to play a central role in precipitating subicular hyperexcitability. While a myriad of mechanisms, including the loss of inhibitory neurons, can result in impaired inhibition, changes in a key chloride ion transporter (potassium–chloride cotransporter; KCC2) have been strongly implicated in TLE.[1,3] A new study by Anstötz et al (Ref.4) now elegantly confirms the sufficiency of subicular KCC2 malfunction to trigger hypersynchronous activity in brain slices.

When considering the circuits of the hippocampal formation, the focus is often on the trisynaptic loop involving the entorhinal cortex, dentate gyrus, and Cornu Ammonis (CA) subfields of the hippocampus proper. However, the subiculum is an additional, key component of the hippocampal formation and one of its primary output structures, processing and modifying inputs from CA1 before transmitting this information onto a number of downstream structures including the nucleus accumbens, thalamus, retrosplenial cortex, and entorhinal cortex.[2,5,6] It is now clear that the connections between CA1 and the subiculum are not just unidirectional, with behaviorally important projections from the subiculum back to CA1.[7] The principal, excitatory neurons of the subiculum can be classified in several distinct ways. Morphologically, these principal neurons are pyramidal cells. Physiologically, they are either bursty or regular-spiking (RS). Functionally, different subsets of subicular neurons can encode distinct navigational information (including speed, place, boundaries, and axis of travel), and neurons with different functions tend to project to distinct target regions.[6,8] Subsets of neurons also play projection-specific roles in the ripple-dependent consolidation of memories to the cortex. Thus, the variation in subiculum principal neuronal physiology and excitability is intricately linked with the function of these neurons, suggesting the existence of multiple parallel circuits but also hinting at highly variable propensity for hyperexcitability in epilepsy.[1]

As in other parts of the hippocampal formation, the activity of subicular principal neurons is tightly regulated by inhibitory inputs utilizing chloride-permeable $GABA_A$ receptors. GABA-mediated chloride influx is critical for successful hyperpolarization or shunting inhibition of the post-synaptic neuron and hence essential for preventing runaway excitation. However, if the intracellular chloride concentration is too high, then activating "inhibitory" synapses leads to the net efflux of chloride ions and corresponding depolarization and excitation of the postsynaptic neuron. KCC2 is one of the cotransporters that is critical for maintaining the appropriate chloride concentration, but it has repeatedly been shown to be dysfunctional in the

subiculum in TLE.[1,3] However, one outstanding question has remained: is the impairment of KCC2 in only the subiculum sufficient to generate hypersynchronous activity similar to that seen in brain slices from TLE patients?

This is the precise question that Anstötz et al[4] answered. They used a reduced brain slice preparation that disconnected the subiculum from other parts of the hippocampus proper and also from the cortex. Using VU0463271, a drug that selectively blocks the KCC2 cotransporter, they showed that KCC2 blockade increased the likelihood of hypersynchronous firing in this isolated subiculum preparation, resembling interictal-like events seen in human epileptic tissue.[1] This was true even in the absence of any other long-term epileptogenic factors, as this tissue was from otherwise nonepileptic mice. If GABA becomes excitatory as a result of KCC2 blockade, then does this mean that the activation of subicular inhibitory neurons can increase the likelihood of interictal-like hypersynchronous network events? Focusing on fast-spiking (FS), parvalbumin-positive neurons that represent one of the largest sources of local inhibition, Anstötz et al confirmed this prediction: Optogenetic activation of FS cells led to an increase in interictal-like events in the presence of the KCC2 blocker, while optogenetic silencing of FS cells decreased the likelihood of interictal-like events. Thus, their experiments convincingly demonstrated the causal ability of "inhibitory" FS cells to initiate hypersynchronous activity in the subiculum that resembled the interictal events seen in tissue resected from human TLE patients.[1] Furthermore, they found that the axonal footprint of individual FS cells roughly matched the area spanned by coactivated principal neurons, again suggesting that FS cells could synchronize the activity of their target principal neurons. This was further supported by the fact that during the interictal network events, FS cell firing preceded that of most principal neurons, further implicating the excitation and synchronization of principal neurons by the initial firing of FS cells, with all of this hyperexcitability made possible by the failure of KCC2 to regulate chloride levels.

The work by Anstötz et al mechanistically confirms years of important work implicating subicular KCC2 failure in TLE.[1-3] Such mechanistic work in simplified preparations is of critical importance in epilepsy research, helping to establish an elemental circuit-level understanding of the sequence of events that can lead to hyperexcitable network states. In intact circuits in TLE patients, feedforward inhibition could easily play the role served by optogenetic excitation of FS cells in the slice experiments. Excitatory inputs from CA1 and other input regions would excite both FS and principal neurons. In the presence of impaired or reduced KCC2, the FS cell firing recruited by CA1 inputs would no longer inhibit subicular principal neurons, but would instead further excite them. Thus, even normal CA1 activity would be able to lead to pathological excitation of the subiculum.

Would all subicular principal neurons be likely to lose KCC2 and become hyperexcitable? The answer is no. Recordings in surgically resected tissue have shown that roughly a quarter of principal neurons lack KCC2 in TLE. It is possible that this subset of neurons corresponds to one of the physiological and/or functional subtypes discussed above, with important implications for what kind of navigational abilities and memories are most likely to be specifically impaired in TLE.

Perhaps one of the most important unresolved questions is why subicular KCC2 expression is lost in the first place. One intriguing possibility is a potential role for amyloid precursor protein (APP). In healthy conditions, the intact form of APP helps to prevent KCC2 degradation via direct physical interactions between the two proteins.[9] Thus, it is possible that abnormal proteolysis of APP (which can lead to β-amyloid peptide, an important feature of Alzheimer's disease pathology) could also impair KCC2 expression. Indeed, the subiculum is also among the most vulnerable brain regions in Alzheimer's disease.[10] The many emerging links between Alzheimer's pathology and the epilepsies could thus potentially include the increased degradation of KCC2 stemming from increased APP proteolysis.

While KCC2 is very strongly implicated in subicular hyperexcitability in TLE, it is not the only known impairment. Inwardly rectifying potassium channels (Kir4.1) in astrocytes and voltage-gated sodium channels in principal neurons, for example, have also been shown to be altered in ways that can promote hypersynchronous subicular activity in TLE. Finally, it is important to note that reduced preparations have inevitable limitations: In particular, the isolated subiculum preparation does not generate long duration ictal events, as carefully discussed in Ref.4. Thus, the combination of mechanistic studies in simplified preparations, animal models of epileptogenesis, and *in vivo* and *ex vivo* human recordings will all continue to be needed to cohesively resolve the precise sequence of events that lead to a hyperexcitable subiculum in TLE.

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