When GABA Fails: Rundown on Chemokines

Fractalkine/CX3CL1 Modulates GABA<sub>A</sub> Currents in Human Temporal Lobe Epilepsy.


PURPOSE: The chemokine fractalkine/CX3CL1 and its receptor CX3CR1 are widely expressed in the central nervous system (CNS). Recent evidence showed that CX3CL1 participates in inflammatory responses that are common features of CNS disorders, such as epilepsy. Mesial temporal lobe epilepsy (MTLE) is the prevalent form of focal epilepsy in adults, and hippocampal sclerosis (HS) represents the most common underlying pathologic abnormality, as demonstrated at autopsy and postresection studies. Relevant features of MTLE are a characteristic pattern of neuronal loss, as are astrogliosis and microglia activation. Several factors affect epileptogenesis in patients with MTLE, including a lack of c-aminobutyric acid (GABA)<sub>A</sub>ergic inhibitory efficacy. Therefore, experiments were designed to investigate whether, in MTLE brain tissues, CX3CL1 may influence GABA<sub>A</sub> receptor (GABA<sub>A</sub>R) mediated transmission, with a particular focus on the action of CX3CL1 on the use-dependent decrease (rundown) of the GABA-evoked currents (IGABA), a feature underlying the reduction of GABAergic function in epileptic tissue. METHODS: Patch-clamp recordings were obtained from cortical pyramidal neurons in slices from six MTLE patients after surgery. Alternatively, the cell membranes from epileptic brain tissues of 17 MTLE patients or from surgical samples and autopsies of nonepileptic patients were microtransplanted into Xenopus oocytes, and IGABA were recorded using the standard two-microelectrode voltage-clamp technique. Immunohistochemical staining and double-labeling studies were carried out on the same brain tissues to analyze CX3CR1 expression. KEY FINDINGS: In native pyramidal neurons from cortical slices of patients with MTLE, CX3CL1 reduced IGABA rundown and affected the recovery of IGABA amplitude from rundown. These same effects were confirmed in oocytes injected with cortical and hippocampal MTLE membranes, whereas CX3CL1 did not influence IGABA in oocytes injected with nonepileptic tissues. Consistent with a specific effect of CX3CL1 on tissues from patients with MTLE, CX3CR1 immunoreactivity was higher in MTLE sclerotic hippocampi than in control tissues, with a prominent expression in activated microglial cells. SIGNIFICANCE: These findings indicate a role for CX3CL1 in MTLE, supporting recent evidence on the relevance of brain inflammation in human epilepsies. Our data demonstrate that in MTLE tissues the reduced GABAergic function can be modulated by CX3CL1. The increased CX3CR1 expression in microglia and the modulation by CX3CL1 of GABAergic currents in human epileptic brain suggest new therapeutic approaches for drug-resistant epilepsies based on the evidence that the propagation of seizures can be influenced by inflammatory processes.

Commentary

A hallmark feature of mesial temporal lobe epilepsy (MTLE) is that endogenous mechanisms that normally limit or reduce neuronal excitability become inefficient over time. Among those, GABA<sub>A</sub> receptor–mediated inhibitory currents have been shown to fail in MTLE, with several mechanisms accounting for this phenomenon, including a reversal of the electrochemical chloride gradient across the plasma membrane (1), altered composition of GABA<sub>A</sub> receptor subunits (2), and the use-dependent decrease (rundown) of GABA-evoked currents (IGABA) (3). The rundown of IGABA is a phenomenon in which the repeated exposure of the receptor to GABA results in a gradual decrease of the resulting currents; IGABA rundown is more pronounced in epileptogenic tissue. GABA<sub>A</sub> receptor function is also influenced by inflammatory processes (4). Because inflammatory processes, and in particular the activation glial cells, play a major role in epileptogenesis, endogenous and exogenous modulators of inflammation are of interest for our understanding of the pathophysiology of epilepsy.

Chemokines are a specific class of small cytokines, which are paracrine messengers secreted by a large variety of cell types, and may trigger chemotaxis in nearby responsive cells. They have been grouped into four families (C, CX3C, CC, and CXC) based on structural motifs. All of these chemokines bind to specific G protein–coupled chemokine receptors. Among the chemokines, several interact with microglial chemokine receptors, modulate inflammatory responses, or have been implicated in epileptogenesis. Mounting evidence demonstrates that several chemokines interact with GABAergic signaling (5).
Thereby, chemokines and their receptors emerge as an interesting class of molecules that have the potential to influence both inflammation and GABAergic signaling, two important targets for the therapy of MTLE. Despite their emerging role as regulators of fundamental processes in the brain, the role of chemokines in epilepsy remains understudied.

The study by Roseti et al. was designed to study a potential role of the chemokine CX3CL1 (fractalkine) and its receptor CX3CR1 in temporal lobe epilepsy. Fractalkine is a chemokine that is prominently upregulated in cerebral cortex, cerebrospinal fluid, and serum of MTLE patients; however, it is not known whether this increase in fractalkine is causally related to the development of epilepsy or merely an adaptive response.

Roseti et al. first studied whether fractalkine could influence the rundown of I_{GABA} in resected tissue from human MTLE patients. Two independent experimental approaches were followed. In the first approach, patch-clamp recordings were performed on cortical pyramidal neurons in slices prepared from resected hippocampi. The second approach made use of an elegant membrane transplantation procedure, in which human membranes were transplanted into Xenopus oocytes. Those humanized oocyte membranes then expressed native human receptors and were used for voltage-clamp recordings. Using both methods, the rundown of I_{GABA} was confirmed in the MTLE samples, but importantly, pretreatment with fractalkine decreased the rundown of I_{GABA} currents in the MTLE-derived preparations, suggesting that fractalkine led to a stabilization of I_{GABA} in the epileptic membrane samples. This effect was specific for MTLE samples, since fractalkine did not influence I_{GABA} in oocytes transplanted with membranes from human control tissues or from resected tissue from patients with cortical dysplasia. These findings suggest that fractalkine might be of therapeutic value in preventing a decline in GABA-responsiveness in MTLE.

In a potential new therapy, it is important to understand how the system in general is regulated in health and disease. Roseti et al. found that the fractalkine receptor CX3CR1 was increased about 6-fold in MTLE in comparison with healthy brain tissue and associated with microglia. Those findings are interesting, because they implicate an association of the CX3CL1/CX3CR1 system with brain inflammatory processes. However, it is not known whether the increased expression of the fractalkine receptor is causally involved in epilepsy development or merely an adaptive response to enable increased fractalkine signaling.

Several open questions remain regarding the underlying mechanisms of fractalkine’s role in epilepsy. If the fractalkine receptor CX3CR1 is overexpressed on microglia, how can the therapeutic use of fractalkine affect I_{GABA}, which is a function of neurons? The efficacy of external fractalkine on I_{GABA} in human slice preparations or humanized oocytes, however, suggests a direct interaction of fractalkine with a certain area of a neuronal membrane. Can CX3CR1 directly interact with GABA_{A} receptors? If this is the case, then GABA_{A} receptors and CX3CR1 would need to be co-localized within the same membrane patch that was transplanted in the oocyte protocol. However, it is currently not clear whether both receptors co-localize or interact in situ.

Additional work is needed to identify the underlying mechanism(s) of fractalkine’s interaction with I_{GABA}. Previous work from the same research group might yield important clues on the underlying mechanism of the findings discussed here. One possibility is that fractalkine triggers effects on neuronal I_{GABA} through a noncell autonomous activity, via activation of microglial CX3CR1. In this case, microglia might be triggered to release a diffusible factor capable of decreasing the rundown of I_{GABA}. Indeed, these researchers demonstrated fractalkine-induced release of adenosine from microglia, which resulted in an inhibitory effect on long-term potentiation (LTP) that could be blocked by exposing hippocampal slices to an adenosine receptor antagonist (6).

In a follow-up study, the adenosine A_{2R} receptor was identified as the key receptor subtype mediating the inhibition of LTP by fractalkine in the CA1 (7), whereas fractalkine-mediated neuroprotection appeared to be dependent on A_{1R} activation (6). In line with those observations, fractalkine induced excitatory postsynaptic current (EPSC) depression in both A_{1R}−/− and/or A_{2AR}−/− mice, but not in A_{3R}−/− mice (8). More recently, the same group demonstrated that fractalkine potentiates the NMDA receptor component of the field excitatory postsynaptic potential (fEPSP) in area CA1 of the hippocampal formation (9). As a mechanistic explanation of this finding, the authors proposed that fractalkine activates CX3CR1 receptors on microglia, leading to the release of adenosine as a paracrine factor, which in turn might activate glial A_{2AR} receptors and trigger the release of D-serine acting as a co-agonist at the NMDA receptor. They previously showed that adenosine can directly influence the stability of GABA_{A} receptors and thereby affect the I_{GABA} rundown (10). Together, these data suggest a fairly complex interplay between inflammatory processes, chemokines and their receptors, paracrine factors released by glial cells, and the stability of I_{GABA}. Identification of the mechanisms that determine the stability of I_{GABA} currents may lead to novel therapeutic concepts for the treatment of MTLE.

by Detlev Boison, PhD

References

5. Caioi S, Pieri M, Antonini A, Guglielmotti A, Severini C, Zona C. Monocyte Chemoattractant Protein-1 upregulates GABA-induced current evidence of modified GABAA subunit composition in cortical neu-


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