Astrocytic NKCC1 inhibits seizures by buffering Cl− and antagonizing neuronal NKCC1 at GABAergic synapses

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論文題目

Astrocytic NKCC1 inhibits seizures by buffering Cl− and antagonizing neuronal NKCC1 at GABAergic synapses

（アストロサイト NKCC1 は GABA 作動性シナプスにおける Cl-緩衝作用で神経細胞 NKCC1 とは拮抗的に痙攣発作を抑制する）

論文の内容の要旨

[Introduction]

Pathological excitatory action of the major inhibitory neurotransmitter γ-aminobutyric acid (GABA) has been observed in epilepsy. Blocking the Cl^- importer Na^+-K^+-$2Cl^-$ type 1 (NKCC1) with bumetanide is expected to reduce the neuronal intracellular Cl^- concentration ($[Cl^-]_i$) and thereby attenuates the excitatory GABA response, so that several clinical trials of bumetanide for treatment of epilepsy are ongoing. Although NKCC1 is expressed not only in neurons but also in glial cells, an involvement of glial NKCC1 in seizures has not yet been elucidated. Astrocytes maintain high $[Cl^-]_i$ with NKCC1 so this gradient promotes Cl^- efflux via astrocytic $GABA_A$ receptor ($GABA_AR$). This Cl^- efflux could buffer the synaptic cleft Cl^- concentration ($[Cl^-]_o$) to maintain the postsynaptic Cl^- gradient during intense firing of GABAergic neurons and so its inhibitory action during seizures. Therefore, we investigated the function of astrocytic NKCC1 in modulating the postsynaptic GABA action in acute seizure models.

[Materials and Methods]

All experiments were approved by the Committee for Animal Care and Use (Approval No. 2019007 and 2-35). We used the astrocyte-specific NKCC1 knockout (AstroNKCC1KO) mice in which the NKCC1 was specifically depleted from astrocytes under the astrocyte promoter *Aldh1l1* in order to reduce the astrocytic $[Cl^-]_i$. The expression of NKCC1 in astrocytes was examined by immunohistochemistry using the antibodies against NKCC1 and Glial fibrillary acidic protein (GFAP). The reduction of astrocytic $[Cl^-]_i$ in AstroNKCC1KO mice was confirmed by examining fluorescence changes of the Cl^- sensitive dye *N*-(ethoxycarbonylmethyl)-6-methoxyquinolinium bromide in response to GABA application. The *in vitro* seizure-like events (SLEs) of hippocampal CA1 pyramidal neurons were triggered by a tetanic electrical stimulation. The SLEs' stimulation intensity threshold was determined by increasing the tetanic stimulation intensity from 50 to 450 μA. The minimal intensity which succeeded in triggering the SLE was considered as the SLE threshold. The duration of SLEs was defined from the ending of stimulation at 450 μA to the time when the membrane potential recovered to the baseline. The activitys of $GABA_AR$ and NKCC1 during SLEs

were inhibited and revealed by application of bicuculline and bumetanide in the perfusing solution, respectively. The *in vivo* pilocarpine-induced acute seizures were monitored in adult mice (aged 3 - 4 months) via the Racine scale.

[Results]

The astrocytic NKCC1 reduction was confirmed by the significant lower ratio of NKCC1 and GFAP co-expression over the total NKCC1 expression in AstroNKCC1KO mice compared to wild-type (WT) littermates. The reduction of NKCC1 in astrocytes consequently reversed the astrocytic $GABA_AR$-mediated Cl^- flux from efflux in WT mice to influx in AstroNKCC1KO mice. The AstroNKCC1KO mice were prone to seizures with lower threshold and longer duration of SLEs, larger $GABA_AR$-mediated depolarization underlying the SLEs, accompanied by the higher Racine-scored seizures *in vivo*. Bumetanide reduced all the above indicatives of higher seizure susceptibility of AstroNKCC1KO mice (with neuronal NKCC1) but not of WT, both *in vitro* and *in vivo*. Thus, astrocytic NKCC1 was preventive in excitatory GABA-mediated seizures ,whereas neuronal one was promotive, suggesting conflicting effects of bumetanide on these cells.

[Discussion]

Conditional depletion of astrocytic NKCC1 in AstroNKCC1KO mice reversed the astrocytic $GABA_AR$ mediated Cl^- flux from outward to inward. It was consistent with the previous results in which pharmacological blocking of NKCC1 by bumetanide significantly reduced astrocytic $[Cl^-]_i$. This confirms NKCC1 plays a crucial role in mediating high astrocytic $[Cl^-]_i$ and render Cl^- efflux via astrocytic $GABA_AR$.

A recent study has been shown the elevation or reduction of astrocytic $[Cl^-]_i$ enhanced or reduced the GABAergic transmission, respectively. We have found that the amplitude of $GABA_AR$-mediated depolarization during SLE was larger in AstroNKCC1KO than in their WT littermates. This indicates that removal of NKCC1 from astrocyte reverses astrocytic $GABA_AR$-mediated Cl^- flux and then exacerbates neuronal $GABA_AR$-mediated depolarization during SLEs. Our results support the notion that astrocytic $[Cl^-]_i$ serves as a source of synaptic cleft Cl^- to sustain inhibitory GABAergic transmission, contributing to ameliorate neuronal Cl^- gradient during SLEs. Altogether, astrocytic NKCC1 plays an epilepsy protective role.

Specific inhibition of the remaining neuronal NKCC1 of the AstroNKCC1KO mice by bumetanide suppressed its seizure susceptibility both *in vitro* and *in vivo*, indicating its anti-seizure action on neuronal NKCC1. However, cell-type nonselective bumetanide did not confer the seizure protection in WT mice, indicating simultaneous inhibition of astrocytic NKCC1 could mask its action on neuronal NKCC1.

[Conclusion]

Our findings suggest a protective role of astrocytic NKCC1 in seizures. This might provide an answer to the question why some of the clinical trials of bumetanide, which is cell-type nonselective, failed to show clear appreciable results.