The change of chloride cotransporters NKCC1 and KCC2 by WNK3 kinase signaling during status epilepticus

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Purpose: GABAergic receptor (GABAR) is involved in maintenance of neuronal chloride (Cl-) homeostasis, and its inhibitory activity induces a low intra-neuronal concentration of Cl-, which is achieved by with no lysine kinase subtype 3 (WNK3) signaling-mediated reversal regulation of Na–K–Cl cotransporter 1 (NKCC1) and K–Cl cotransporter 2 (KCC2). We examined the dynamic alterations of NKCC1 and KCC2 by inhibition of WNK3 signaling during prolonged status epilepticus (SE) to identify their role in the benzodiazepine resistance of the refractory SE. Method: For induction of SE, we used in vitro magnesium (Mg2+)-free cultured neuron model of SE and in vivo mouse model of pilocarpine-induced SE. Biotinylation assay and western blot analysis were performed to monitor the changes of total and phosphorylated NKCC1 and KCC2 in Mg2+-free cultured neurons. To examine the changes of NKCC1 and KCC2 following inhibition of WNK3 signaling, compound B, known as WNK signaling inhibitor that was treated after SE induction, and its effects were examined by immunofluorescent staining and western blot analysis. Results: In the cultured neurons, expression of membrane KCC2 gradually disappeared after SE; in contract, enhanced immunoreactivities of membrane NKCC1 were observed over time after SE. Western blotting indicated a significant decrease in the levels of membrane KCC2 during prolonged SE, suggesting that the internalization of KCC2 might be exerted by prolonged SE. However, inhibition of WNK3 signaling following treatment with compound B promoted the upregulation of membrane KCC2 in Mg2+-free cultured neurons, and induced a significant increase in the levels of phosphorylated KCC2 after pilocarpine-induced SE in vivo. Conclusion: During prolonged SE, WNK3 signaling contributed to the dynamic alteration of NKCC1 and KCC2 expression, which might affect inhibitory activity of GABAR after SE. These results suggest that controlling of WNK3 signaling pathway may be a potential therapeutic target for benzodiazepine-refractory SE.