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Influence of Maternal High Salt Diet on Fetal Brain Development Via Osmotic and Na, Cl Modulators

Atsuo Fukuda[1], Tenpei Akita[1], Miho Watanabe[1], Hiroki Mutoh[1], Kenichiro Hata[2]

[1] Department of neurophysiology, Hamamatsu University School of Medicine

[2] Department of Maternal-Fetal Biology, National Research Institute for Child Health and Development

Summary

We are interested in the Cl^- homeodynamics and multimodal GABA actions. We used WNK3 knock-out (KO) mice, since WNK family kinases are essential elements in the signaling cascade regulating Cl^- concentrations after salt intake. WNK family kinases are known to phosphorylate KCC2 at two threonine (Thr^{906} and Thr^{1007}) residues via downstream kinases, SPAK/OSR1. So, we engineered mice with the missense mutations Glu^{906} and Glu^{1007} (*Kcc2*$^{e/e}$) to mimic constitutive phosphorylation. Intracellular taurine imported by taurine transporter (TauT) activate the signaling cascade of WNK1 and SPAK/OSR1, hence we also used TauT KO mice.

In TauT KO mice, the amplitude of miniature inhibitory postsynaptic currents decreased. Although dose-response of $GABA_A$ receptor (R) indicated no differences in sensitivity, the maximum currents and γ2 subunit immunostaining were significantly decreased, indicating reduced numbers of postsynaptic $GABA_A$Rs in TauT KO mice. In WNK3 KO, neuronal excitability was significantly reduced, e.g., hyperpolarized resting membrane potential (RMP), decreases in input resistance and membrane time constant, and resultant increase in the action potential threshold current. Inwardly rectifying potassium conductance (IRK) underlying RMP was enhanced and injection of WNK3 to recorded neurons restored this enhancement. Thus, WNK3 regulates IRK via phosphorylation of downstream kinases, e.g., SPAK/OSR1. Phosphorylation of KCC2 at Thr^{906} and Thr^{1007}, which inhibits KCC2 activity, decreases with an increase in KCC2 activity and the lowering of neuronal $[Cl^-]_i$ during brain development. *KCC2*$^{e/e}$ mice demonstrated abnormal neuronal distribution, status epilepticus provoked by mild physiological stimulation, normal resting $[Cl^-]_i$ but with significantly impaired Cl^- extrusion capacity after Cl^- loading, a lack of spontaneous respiratory discharge and an altered locomotor rhythm. Thus, precisely regulated KCC2 Thr^{906}/Thr^{1007} phosphorylation is essential for activity-dependent Cl^- extrusion required for normal brain development.