Insights into Lithium Interaction with the Na⁺/Cl⁻-dependent GABA cotransporter.

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Introduction: Li⁺ can interact with the Na⁺/Cl⁻ dependent GABA transporter, GAT-1: Li⁺ induces a voltage gated leak current in the absence of Na⁺; in the presence of Na⁺ and GABA, Li⁺ affects the apparent affinity for Na⁺ (MacAulay et al. J Physiol. 2002 vol 544, Kanner JBC 2003, vol 278). Despite the fact that the amino acids directly involved in the interaction with the Na⁺ and Li⁺ ions in the Na2 binding site have been identified (Zhou et al. JBC 2006, vol 281), it is still not clear how Li⁺ affects the kinetics of cotransport. Here, we provide evidence that when Li⁺ is present together with Na⁺, a fraction of the transporters will be cotransporting Li⁺, Na⁺ and GABA; and a fraction 2 Na⁺ and 1 GABA, and that Li⁺ interaction changes specific rate constants of the transport cycle.

Methods: rat GAT-1 was expressed in Xenopus laevis oocytes and two-electrode voltage clamp electrophysiology and radioactive tracer uptake experiments under voltage clamped conditions were performed in order to investigate the effect of external Li⁺ on transport kinetics.

Results: Steady-state analysis of GABA-induced currents revealed a reduced apparent affinity for GABA (K_{0.5}^{GABA}) that was depending on the amount of Li⁺ substituting for Na⁺, however at saturation [GABA] the maximum transport rate (I_{max}) was not affected by Li⁺. Na⁺ activation experiments, where Na⁺ was either equimolarly exchanged with Li⁺ or choline and at saturation [GABA], revealed apparent Na⁺ affinities (K_{0.5}^{Na}) in the micromolar range (500 to 1000 µM), a Hill coefficient less than one and a voltage dependent I_{max} when Li⁺ was the substituting ion, whereas when Na⁺ was substituted with choline, the K_{0.5}^{Na} was in the mM range (20-500 mM), the Hill coefficient was >1 and I_{max} was voltage independent. By means of ³²Na-uptake experiments under voltage clamped conditions we showed that Li⁺ can substitute for, and are translocated like, one of the 2 Na⁺ ions that participate in cotransport. We performed presteady-state assays to identify which partial reactions in the transport cycle that was affected by Li⁺ interactions. Effectively, we found that Li⁺ increase the relaxations time constants of the presteady-state relaxations and further more reduces the shift of the V_{0.5} towards more negative potentials caused by lowering the [Na⁺] by substituting with Li⁺ compared to choline.

Interpretation: We propose an ordered binding scheme for cotransport in which either a Na⁺ or Li⁺ ion can bind at the putative 1st cation binding site. This is followed by the cooperative binding of the second Na⁺ ion and then GABA. With Li⁺ bound in the first “low affine” binding site, the 2nd Na⁺ ion is more readily bound to the protein, and despite the lower GABA affinity, the translocation rate of the fully loaded carrier is not reduced. Model simulations will confirm our experimental findings.

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