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Expression of the NKCC2A Cotransporter in Mouse Central Nervous System

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Introduction

Na-K-2Cl cotransporter 2A (NKCC2A), also known as the bumetanide-sensitive cotransporter 1 (BSC1), transports Na⁺, K⁺ and Cl⁻ with a stoichiometry of 1:1:2. NKCC2A is considered a kidney specific cotransporter. It is abundantly expressed in apical membrane of the tubular cells in the thick ascending limb of the loop of Henle (TALH) and in the macula densa. However, NKCC2 has also been found at low levels in different cells, including insulin-secreting ones. This secondary active transporter uses the energy stored in the electrochemical gradients of Na and K maintained by the Na/K-ATPase located on the basolateral membrane of the TALH. The gene encoding NKCC2 is *Slc12a1*, solute carrier family 12 member 1 located on chromosome 2. Mice lacking NKCC2A (NKCC2A-KO) exhibit mild kidney dysfunction. In this lab it was noted that NKCC2A-KO mice express atypical muscle movements, slight tremors and subtle muscle dyscontrol, endure exceptionally well in the forced swim test and had changes in neurotransmitter levels. These observations led to the hypothesis that NKCC2A may be also found in the central nervous system (CNS).

Objective: Determine if NKCC2A is expressed in the mouse brain

Materials and Methods

Animals

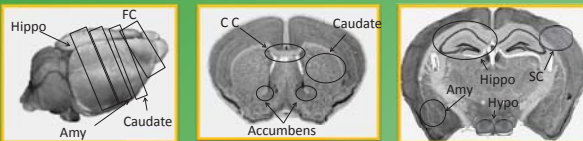
- 2 month old C57BL/6J mice (Wild Type mice, NKCC2-KO and Heterozygous mice)

Histology

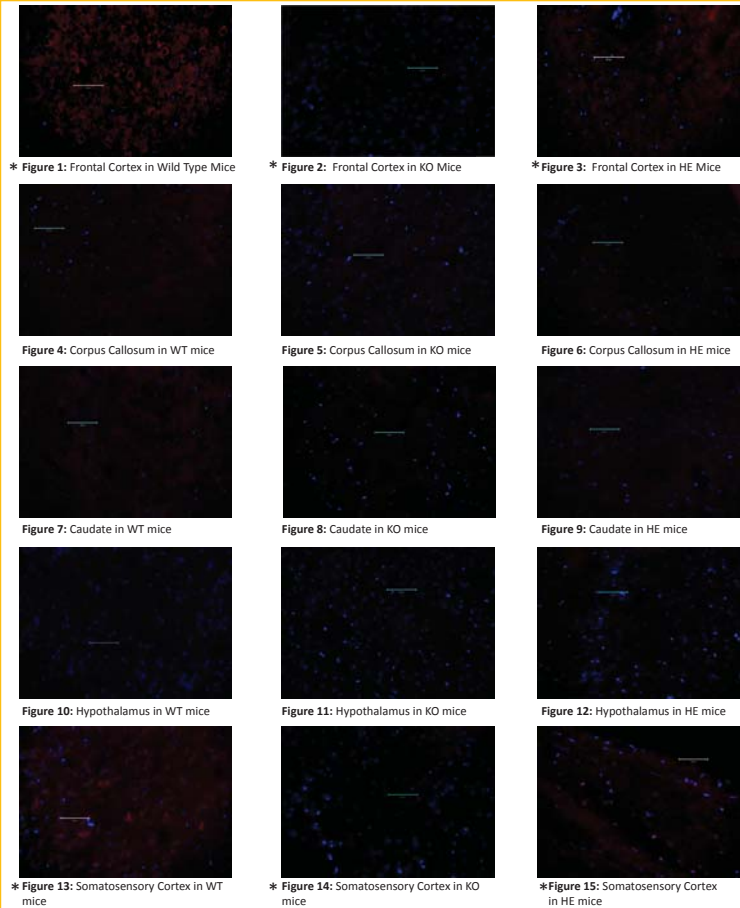
- 15 micron coronal sections obtained from interaural 6.76 mm to 5.9 mm, 5.34 mm to 4.54 mm, and 2.68 mm to 1.62 mm

Immunofluorescence Staining

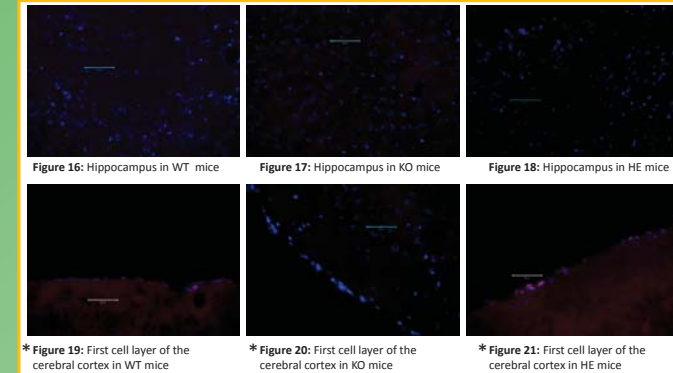
- Specific staining for NKCC2 channel, red and cell nuclei, blue
- Images were taken at 40x of the frontal cortex (FC), corpus callosum (CC), caudate, accumbens, hypothalamus (Hypo), amygdala (Amy), somatosensory cortex(SC) and hippocampus (Hippo)



Results



Results



- Positive NKCC2A staining was observed in: frontal cortex, somatosensory cortex, and the first cell layer of the cerebral cortex, but not positive in corpus callosum, caudate, accumbens, hypothalamus, amygdala and hippocampus
- NKCC2 expression was noted to be intracellular
- Expression of NKCC2 was much higher in wild type mice compared to heterozygous mice. No expression of NKCC2 was observed in knockout mice.
- Positive staining for NKCC2 was noted in Kidney control.
- When *SLC12A1* blocking peptide was applied to wild type tissue, no positive staining for NKCC2 was observed.

Conclusion

- These data reveal that NKCC2A is located in specific regions of the mouse brain which may help explaining the changes in behavior and neurochemistry observed in NKCC2A-KO mice.

Acknowledgements

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