

cAMP Stimulates NKCC2 Insertion and Trafficking via PKA in the Thick Ascending Limb

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The Na/K/2Cl co-transporter NKCC2 plays a crucial role in water and electrolyte balance by mediating NaCl absorption in the thick ascending limb (TAL) of Henle's loop. cAMP stimulates surface NKCC2 expression and NaCl absorption via NKCC2 in the TAL. However, little is known about the regulation of NKCC2 trafficking by cAMP. We hypothesize that cAMP stimulates surface NKCC2 expression via PKA in TALs. We also hypothesize that the primary trafficking process is exocytic insertion, and it is mediated by PKA. We used surface biotinylation in rat TAL suspensions to measure surface NKCC2 levels and a modified surface biotinylation protocol to measure NKCC2 exocytic insertion. We increased cAMP levels with 20 μ M Forskolin + 0.5 mM IBMX and observed an 81 % increase in surface NKCC2. The PKA inhibitor H-89 (10 μ M) blocked 70 % of this effect. We found that stimulation of V2 receptors in TAL with 1 μ M dDAVP + 0.5 mM IBMX produced an increase of 44 % in surface NKCC2 levels compared to basal (untreated). This increase was completely blocked by pre-treatment with H-89. We also observed constitutive NKCC2 exocytic insertion at 15, 30 and 45 minutes. Forskolin+IBMX also increased NKCC2 insertion and H-89 blocked 60 % of this effect without affecting basal NKCC2 insertion. In conclusion, our data suggest that cAMP and V2 receptors stimulation increases surface NKCC2 expression in TALs via PKA. We also report evidence of constitutive NKCC2 insertion in the plasma membrane, which is not dependent on PKA. However, cAMP stimulates NKCC2 exocytic insertion via PKA.

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