Regulation of the Na(+)-K(+)-2Cl(-) cotransporter by cGMP/cGMP-dependent protein kinase I after furosemide administration.

Sodium chloride reabsorption in the thick ascending limb of the loop of Henle is mediated by the Na(+)-K(+)-2Cl(-) cotransporter (NKCC2). The loop diuretic furosemide is a potent inhibitor of NKCC2. However, less is known about the mechanism regulating the electrolyte transporter. Considering the well-established effects of nitric oxide on NKCC2 activity, cGMP is likely involved in this regulation. cGMP-dependent protein kinase I (cGKI; PKGI) is a cGMP target protein that phosphorylates different substrates after activation through cGMP. We investigated the potential correlation between the cGMP/cGKI pathway and NKCC2 regulation. We treated wild-type (wt) and cGKI?-rescue mice with furosemide. cGKI?-rescue mice expressed cGKI? only under the control of the smooth muscle-specific transgelin (SM22) promoter in a cGKI deficient background. Furosemide treatment increased the urine excretion of sodium and chloride in cGKI?-rescue mice compared to that in wt mice. We analyzed the phosphorylation of NKCC2 by western blotting and immunostaining using the phosphospecific antibody R5. The administration of furosemide significantly increased the phosphorylated NKCC2 signal in wt but not in cGKI?-rescue mice. NKCC2
activation led to its phosphorylation and membrane translocation. To examine whether cGKI was involved in this process, we analyzed vasodilator-stimulated phosphoprotein, which is phosphorylated by cGKI. Furosemide injection resulted in increased vasodilator-stimulated phosphoprotein phosphorylation in wt mice. We hypothesize that furosemide administration activated cGKI, leading to NKCC2 phosphorylation and membrane translocation. This cGKI-mediated pathway could be a mechanism to compensate for the inhibitory effect of furosemide on NKCC2.