Parathyroid hormone (PTH) regulates the sodium chloride cotransporter via Ras guanyl releasing protein 1 (Ras-GRP1) and extracellular signal-regulated kinase (ERK)1/2 mitogen-activated protein kinase (MAPK) pathway

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The sodium chloride cotransporter (NCC) is the principal salt absorptive pathway in the mammalian distal convoluted tubule (DCT) and is the site of action of thiazide diuretics. Using a mammalian cell model system to assess NCC function, we demonstrated previously that Ras guanyl releasing protein 1 (Ras-GRP1) mediates phorbol ester-induced suppression of the function and surface expression of NCC in a protein kinase C (PKC)-independent and extracellular signal-regulated kinase (ERK)1/2-dependent manner. Given that phorbol esters are functional analogs of diacylglycerol (DAG), this finding suggested a potential physiologic regulation of NCC by DAG. The parathyroid hormone (PTH) receptor is a G-protein-coupled receptor that is expressed in the DCT and activates PLC resulting in the generation of DAG. In this article, we demonstrate that PTH suppresses NCC function via a PLC/Ras-GRP1/ERK pathway. A functional assessment of NCC measuring thiazide-sensitive $^{22}Na^{+}$ flux revealed that PTH suppresses NCC function. The inhibition of PLC prevented the suppression of NCC, indicating that PLC was necessary for this effect. Inhibitors of PKC and protein kinase A (PKA) had no effect on this suppression, but mitogen-activated protein kinase (MAPK) inhibitors prevented the PTH effect completely. Ras-GRP1 activates the MAPK pathway though activation of the small G-protein Ras. Gene silencing of Ras-GRP1 prevented the PTH-mediated suppression of NCC activity, the activation of the H-Ras isoform of Ras, and the activation of ERK1/2 MAPK. This finding confirmed the critical role of Ras-GRP1 in mediating the PTH-induced suppression of NCC activity through stimulation of the MAPK pathway. (Translational Research 2011;158:282–289)

Abbreviations: DAG = diacylglycerol; DCT = distal convoluted tubule; DMEM = Dulbecco's modified eagle's medium; FBS = fetal bovine serum; GPCR = G-protein-coupled receptor; IgG = immunoglobulin G; mDCT = mouse distal convoluted tubule; NCC = sodium chloride cotransporter; PAGE = polyacrylamide gel electrophoresis; PKA = protein kinase A; PKC = protein kinase C; PLC = phospholipase C; PTH = parathyroid hormone; Ras-GRP1 = Ras guanyl releasing protein 1; SDS = sodium dodecyl sulfate; siRNA = small interfering RNA; TBST = tris-buffered saline and Tween 20

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AT A GLANCE COMMENTARY

Ko B, et al.

Background

The sodium chloride cotransporter (NCC) is the principal salt absorptive pathway in the distal convoluted tubule (DCT). We demonstrated previously that Ras guanyl-releasing protein 1 (Ras-GRP1) mediates phorbol ester-induced suppression of NCC function. In this study, we demonstrate that parathyroid hormone (PTH) suppresses NCC function via this pathway.

Translational Significance

This study may help to explain the diuretic effect of PTH. Furthermore, changes in NCC activity result in changes in DCT calcium handling. Therefore, a role for PTH in regulating NCC may be another mechanism by which PTH regulates calcium. An understanding of this may ultimately provide assistance in the management of hyperparathyroidism.

The thiazide-sensitive sodium-chloride cotransporter (NCC) is the salt reabsorptive pathway localized to the apical membrane of the mammalian distal convoluted tubule (DCT) that is responsible for reabsorbing 5% to 10% of the filtered load of sodium.[1] Pharmacologic inhibition of NCC by thiazide diuretics decreases blood pressure and increases calcium reabsorption in the kidney.[2-4] NCC has also been shown to play a role in genetic disorders of calcium handling, hypotension, and hypertension.[5-7] Numerous studies demonstrated that alterations of the function of NCC result in changes in the handling of both calcium and sodium.[3,4,7,8] Despite the importance of this cotransporter in human disease, the hormonal regulation of this cotransporter in the mammalian kidney has not been studied comprehensively. The relative difficulty in isolating the DCT for microperfusion studies and the lack of a mammalian DCT cell line that is amenable to physiologic studies have been the primary hindrances to investigating the regulation of this cotransporter.

Recently, we established such a model and used it to examine the suppression of NCC function by diacylglycerol (DAG) analogs (phorbol esters) through the activation of Ras guanyl releasing protein 1 (Ras-GRP1).[9] Interestingly, this effect of phorbol esters did not involve protein kinase C (PKC). Phorbol esters and DAG can bind and activate 5 different families of proteins, including the Ras-GRP family of proteins.[10] Phorbol esters seem to mediate the suppression of NCC through the activation of Ras-GRP1, resulting in the activation of Ras.[9] This action triggers the Raf/mitogen-activated protein kinase or extracellular signal-related kinase (MEK)/extracellular signal-regulated kinase (ERK) mitogen-activated protein kinase (MAPK) cascade of kinases ultimately resulting in decreased surface expression of NCC.[9] These studies were the first steps toward modeling the physiologic regulation of NCC by DAG. However, although phorbol esters bind and activate the same 5 families of proteins to which DAG binds, they are not metabolized like DAG; they lack the appropriate negative feedback mechanisms. Additionally, they bypass the physiologic pathway of an associated hormone, which traditionally would bind a G protein–coupled receptor (GPCR), activating phospholipase C (PLC) and releasing DAG. Parathyroid hormone (PTH) is a GPCR present in the DCT and known to stimulate the ERK 1/2 MAPK pathway.[11-16] PTH is known to trigger a proximal tubule acute diuretic effect, and most notably, it regulates calcium reabsorption in the DCT, which is a known site of uncoupled calcium and sodium transport.[17-19] We theorized that this uncoupling of sodium transport from calcium reabsorption observed in the DCT could be caused partly by the action of PTH on NCC. Therefore, we examined the regulation of NCC by PTH. We present evidence that PTH suppresses NCC function and surface expression through DAG activation of Ras-GRP1 and the ERK1/2 MAPK pathway.

METHODS AND MATERIALS

Cell culture. Mouse distal convoluted tubule (mDCT) cells (gift of Peter Friedman) were plated on 100-mm cell culture plates and grown to the desired confluence in growth medium containing 50:50 mix of Dulbecco's modified eagle's medium (DMEM)/F12, 5% (vol/vol) heat-inactivated fetal bovine serum (FBS) and 1% penicillin/streptomycin/neomycin (P/S/N) (50 units/mL penicillin, 50 μg/mL streptomycin, 100 μg/mL neomycin), at 37°C.

Assessment of NCC function in mDCT cells. mDCT cells were seeded in 12-well plates and grown to approximately 90% confluence in a medium containing a 50:50 mix of DMEM/F12, 5% heat-inactivated FBS, and 1% P/S/N. The cells were then incubated in a serum-free, preuptake medium (130 mmol/L Na Gluconate, 2 mmol/L K Gluconate, 1.0 mmol/L Ca Gluconate, 1mmol/L Mg Gluconate, 5 mmol/L HEPES /Tris pH 7.4, 1 mmol/L amiloride, 0.1 mmol/L bumetanide) for 30 min. During this time period, the cells were treated with the indicated concentration of PTH. The