Trafficking of ENaC subunits in response to acute insulin in mouse kidney

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Tiwari S, Nordquist L, Halagappa VK, Ecelbarger CA. Trafficking of ENaC subunits in response to acute insulin in mouse kidney. Am J Physiol Renal Physiol 293: F178–F185, 2007. First published March 27, 2007; doi:10.1152/ajprenal.00447.2006.—Studies done in cell culture have demonstrated that insulin activates the epithelial sodium channel (ENaC) via a variety of mechanisms. However, to date, upregulation of ENaC in native renal tissue by in vivo administration of insulin has not been demonstrated. To address this, we injected 6-mo-old male C57BL/6J mice (n = 14/group) intraperitoneally with vehicle or 0.5 U/kg body wt insulin and examined short-term (1–2 h) sodium excretion and kidney ENaC subunits (α, β, and γ) and serum and glucocorticoid-induced kinase (SGK-1) regulation. Insulin resulted in a significant reduction in urinary sodium (by ~80%) that was restored by intraperitoneal administration of the ENaC antagonist, benzamil (1.4 mg/kg body wt). Differential centrifugation followed by Western blotting of whole kidney revealed significantly increased band densities (by 26–103%) for insulin–relative to vehicle-treated mice for α- and γ-ENaC in the homogenate (H), and plasma membrane-enriched fraction (MF), with no difference in the vesicle-enriched fraction (VF). Similarly, β-ENaC was significantly increased in MF (by 45%) but no change in the H. It was, however, significantly decreased in the VF (by 28%) with insulin. In agreement, immunoperoxidase labeling demonstrated relatively stronger apical, relative to cytosolic, localization of α-, β-, and γ-ENaC with insulin, whereas, with vehicle, labeling was fairly evenly dispersed throughout collecting duct principal cells. Furthermore, Western blotting showed insulin increased SGK-1 (by 75%) and phosphorylated-SGK band densities (by 30%) but only in the MF. These studies demonstrate novel in vivo regulation of renal ENaC activity and subunit proteins and SGK-1 by insulin in the acute time frame in the mouse.

epithelial sodium channel; SGK-1; hyperinsulinemia

INSULIN HAS BEEN SHOWN to have antinatriuretic properties (32). Early studies by Atchley et al. (4) demonstrated that discontinuation of an insulin infusion to diabetic patients resulted in a brisk natriuresis. Studies in dogs and humans have shown that insulin increased sodium reabsorption by the kidney, as well as reduced sodium excretion, independently of blood glucose levels, filtered load of glucose, glomerular filtration rate, renal blood flow, and plasma aldosterone levels (11, 12). Thus this peptide hormone may play a role in the development of the hypertension associated with Syndrome X, or risk factor clustering, a common age-related syndrome that is expressed as hyperinsulinemia, obesity, hypertension, and lipid abnormalities.

The kidney regulates salt and water balance in the body through the modification of the activity of sodium transporters and channels expressed along the length of the renal tubule. Micropuncture and perfused tubule studies have revealed that insulin increases sodium reabsorption in the proximal tubule (5, 18, 19), the thick ascending limb (26, 28, 30), and in the distal tubule or the collecting duct (12, 20).

The multimeric, amiloride-sensitive epithelial sodium channel (ENaC) is a rate-limiting step for sodium transport across tight epithelia. Located in the apical membrane of the late distal convoluted tubule through the collecting duct cells, ENaC constitutes a key factor in the maintenance of sodium homeostasis. Studies done on the sodium transport system of the toad bladder (24), in cell culture using the A6-cell line derived from Xenopus laevis kidney (7, 8, 38), and mammalian mpkCCD (cl4) cells (41) have revealed that insulin positively regulates the activity of the ENaC. However, data from in vivo studies utilizing native tissue are lacking.

Serum and glucocorticoid-regulated kinase-1 (SGK-1) has been shown to positively regulate the activity of ENaC (10). SGK-1 may also mediate the effects of hormones such as aldosterone, vasopressin, and insulin on ENaC (1, 16, 27, 35, 36). Furthermore, experiments in Caenorhabditis elegans have demonstrated that SGK-1 is important for insulin signaling via phosphorylation (39). More recently, Huang et al. (25) showed that SGK-1 plays an important role in insulin-mediated sodium retention using SGK-1 knockout mice.

The regulation of ENaC activity depends on the abundance of the protein in the apical membrane, i.e., trafficking to and stability at the cell surface. ENaC trafficking is regulated, both at the level of ENaC movement to the cell surface, as well as ENaC endocytosis and degradation. Previously, we showed that chronic insulin infusion to Sprague-Dawley rats resulted in relatively more apical localization of ENaC subunits (45), compared with vehicle-treated rats, without any change in the total abundance of the subunits at the protein level. However, no one, to our knowledge, has determined whether short-term elevations in circulating insulin levels in vivo result in redistribution of ENaC subunits in kidney tissue and whether this is associated with antinatriuresis and regulation of SGK-1. In this study, we aimed to investigate the effects of hyperinsulinemia in the acute time frame on ENaC and SGK-1 regulation, as well as sodium excretion. In this acute (1- to 2-h period), fewer adaptive changes would be expected to occur in the kidney to counter sodium retention, at least involving major remodeling of the renal tubule.

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METHODS

Animal model of acute hyperinsulinemia. Male, 6-mo-old C57BL/CBA mice from our own colony (Georgetown University) were administered either vehicle or insulin (Humulin) 0.5 U/kg body wt, as a single intraperitoneal injection in 300 μl of sterile 0.9% NaCl (saline). Insulin-treated mice (n = 8) also received a second intraperitoneal injection of 300 μl of 25% dextrose in saline. Vehicle-treated mice (n = 8) received 600 μl of saline. Blood glucose was measured at 30 and 60 min following the injections in tail blood by glucometer (TheraSense, Alameda, CA). At the end of 60 min, mice were deeply anesthetized with pentobarbital sodium, the abdominal cavity was opened, and the left kidney was perfused through the left ventricle with 2% paraformaldehyde in buffer (51). Before the perfusion, the right kidney was clamped off, removed, and prepared for Western blotting as described below. Mice expired during this procedure.

Role of ENaC in antinatriuretic actions of insulin. To test the role of ENaC in antinatriuretic actions of insulin, two experiments were performed. In the first experiment, mice (same age, sex, and strain as above) were injected with insulin and 25% dextrose or vehicle (n = 6/treatment) and urine was collected for 2.5 h. Three days later, all the mice in the above set were administered benzamil (1.4 mg/kg body wt ip; Sigma, St. Louis, MO), a specific antagonist of ENaC, 30 min following insulin-plus-dextrose or vehicle injections, as above, and then urine was collected for 2.5 h. The 30-min lag period was instituted to allow time for ENaC to be upregulated by insulin before antagonizing its actions. The antinatriuretic response to benzamil has been used as an index of relative ENaC activity (22) and was dosed based on a previously published therapeutic dose (40).

The second experiment was the same as the first except that the benzamil was given to all mice 30 min before the insulin-plus-dextrose or vehicle. Urinary sodium was measured by an ion-selective electrode system (ELISE Electrolyte System, Beckman Instruments, Brea, CA).

Preparation of membrane fractions. Right kidneys were removed from mice and homogenized using a tissue homogenizer (Omni 2000 fitted with a 7- or 10-mm micro-sawtooth generator) in 5 ml chilled isolation buffer containing 250 mM sucrose, 10 mM triethanolamine (Calbiochem, La Jolla, CA), 1 μg/ml leupeptin (Bachem, Torrance, CA), and 0.1 μg/ml phenylmethylsulfonyl fluoride (US Biochemical, Toledo, OH) adjusted to pH 7.6 with 1 N NaOH. An aliquot of the homogenate (H) was used to analyze whole kidney abundance of the proteins. The rest of the homogenate was subjected to subcellular fractionation as described by Mandon et al. (29) to obtain a high-density, plasma membrane-enriched fraction (MF) and a low-density, intracellular vesicle-enriched fraction (VF). Briefly, first low-speed centrifugation was performed at 17,000 g for 20 min to obtain a pellet (15). The supernatant (S1) obtained was saved while the pellet was resuspended in 1 ml of isolation buffer, rehomogenized, and again subjected to centrifugation at 17,000 g for 20 min. The pellet obtained (high-density membrane fraction, MF) was retained, and the supernatant (S2) combined with the previous one, S1. The pooled supernatants were then centrifuged at 200,000 g for 1 h to obtain the low-density, vesicle-enriched fraction (VF) as a pellet. The high-speed supernatant was discarded. MF and VF pellets were resuspended in a small amount of isolation solution. Pellets were resuspended by vigorous pipetting and vortexing so that the solution obtained, upon careful inspection, appeared free of floating chunks. Protein concentrations of these suspensions (MF and VF) and the homogenate (H) were determined by the BCA protein assay (Pierce, Rockford, IL). All samples were then diluted with isolation solution to a final protein concentration of 1 μg/μl and solubilized at 60°C for 15 min in Laemmli sample buffer. Samples were stored at −80°C until ready to run on gels. As previously documented, the low-density membrane fraction from this protocol is virtually devoid of plasma membranes (15). We confirmed this by blotting these fractions with antibodies against the α-1 subunit of Na-K-ATPase (membrane-specific protein), GAPDH (glyceraldehyde-3-phosphatase), cytosolic-specific protein (33), and syntaxin-4 (apical membrane marker for renal collecting duct cells) (29).

Electrophoresis and blotting of membranes. Initially, Coomassie-stained loading gels were prepared and densitometry scanned for all sample sets to assess the quality of the proteins by the sharpness of the bands and to confirm precision of protein concentration measurements, as previously described (13, 14). Small adjustments were made in the protein concentrations to reflect what was observed on these loading gels. For immunoblotting, 5–30 μg of protein from each sample were loaded into individual lanes of precast minigels of 7, 10, or 12% polyacrylamide (Bio-Rad, Hercules, CA). Our immunoblotting protocol and the production, affinity purification, and characterization of the polyclonal antibodies against α-, β-, and γ-ENaC have been previously described (44). SGK-1 was a sheep polyclonal obtained from Upstate Biotechnology (Lake Placid, NY). The phosphorylated and nonphosphorylated forms of SGK were distinguished by their differential mobilities (34) and by the use of a p-SGK-1-specific antibody obtained from Santa Cruz Biotechnology (Santa Cruz, CA). We used mouse monoclonal antibodies against the α1-subunit of Na-K-ATPase (Upstate Biotechnology) and GAPDH (Chemicon, Temecula, CA) and a rabbit polyclonal antibody against syntaxin-4 (Calbiochem) to evaluate the purity of fractions prepared in this study.

Immunohistochemistry. Perfusion-fixed left kidneys were used for immunohistochemical examination of the ENaC subunits. The left kidney was processed to paraffin and 5-μm sections were cut. Heat-induced target retrieval was performed using a citrate buffer, pH 6 (Zymed Laboratories), to unmask antigenic sites. Endogenous peroxidase activity was removed by incubation with 2% H2O2 for 20 min. Tissues were incubated with the primary antibodies (1:1,000), i.e., α-, β-, and γ-ENaC overnight at 4°C. The Envision + System (DakoCytomation, Carpinteria, CA) goat anti-rabbit antibody was used to conduct peroxidase labeling. 3,3'-Diaminobenzidine tetra-chloride dihydrate (DAB) was applied for 3–5 min and the tissue was counterstained with Mayer’s hematoxylin to allow anatomical definition. A positive reaction was identified as a brown stain in the cytoplasm. Pictures were taken with a Photometrics Cool Snap camera (ScancoSciences, Fairfax, VA) mounted to a Nikon Eclipse E600 microscope with a ×100 oil-immersion lens for a total magnification of ×1,000. A semiquantitative analysis was done by using Adobe Photoshop-6 as described by Bishop et al. (6). Briefly, the region of the apical cell membrane was selected and the luminosity value was obtained for each collecting duct principal cells. Luminosity has a range of 256 levels, with “0” being the darkest possible color (black) and “255” being the lightest possible color (white). Thus the lower the luminosity level, the more intense is the staining. We used the formula intensity = 1/luminosity to calculate the mean intensity value. Similarly, mean intensity of ENaC subunits staining was obtained for cytoplasmic regions. Sections from five mice per group were analyzed.

Statistical analysis. Data were evaluated using Sigma Stat software (Chicago, IL). Data are presented as means ± SE. The unpaired t-test was used to determine significant differences between pairs of means when animals were different between the two treatments. The paired t-test was used to determine differences between means when the same animals were studied under two conditions. P < 0.05 was considered significant for all analyses.

RESULTS

Blood glucose. Blood glucose was significantly reduced by insulin-plus-dextrose at 30 min relative to vehicle, but mice were not hypoglycemic. Their blood glucose levels at 30 and 60 min, respectively, were (in mmol/l) 11.3 ± 0.8 (vehicle) vs.
7.3 ± 1 (insulin-plus-dextrose) and 9.6 ± 0.7 (vehicle) vs. 8.1 ± 0.9 (insulin-plus-dextrose).

Antinatriuretic effect of insulin and its blockade with benzamil. Urinary sodium excretion was significantly lower when mice were treated with insulin-plus-dextrose relative to when they were treated with vehicle in urine collected in the 2.5 h following treatments (left bars in Fig. 1, A and B), confirming the role of insulin as an antinatriuretic agent. Urine volume was modestly yet significantly decreased in insulin-plus-dextrose-treated mice relative to vehicle (P = 0.03). The mean urine volumes (ml/2.5 h) for insulin-plus-dextrose and vehicle groups were 0.52 ± 0.07 and 0.77 ± 0.10, respectively.

Benzamil (ENaC antagonist) administered 30 min following (Fig. 1A) or before (Fig. 1B) insulin-plus-dextrose or vehicle treatments was used to determine how much of the antinatriuretic effect of insulin might be attributable to increased ENaC activity. When benzamil was administered following the treatments, there was an increase in urinary sodium excretion in both groups (Fig. 1A) and the apparent difference between treatments was no longer significant. When benzamil was administered before the treatments, antinatriuresis due to insulin was completely blocked (Fig. 1B).

Purity of kidney fractions. Band density specific for Na-K-ATPase, a plasma membrane-associated protein, was observed only in the MF prepared from the kidney, while that for GAPDH, an intracellular protein, was observed only in the intracellular vesicle-enriched fraction (VF), confirming the purity of the fractions prepared in this study (Fig. 2). Furthermore, we also found abundant expression of syntaxin-4 in the

Fig. 1. Role of epithelial sodium channel (ENaC) in the antinatriuretic effect of insulin. A: mice were injected with vehicle or insulin plus dextrose (n = 6/group) and placed in metabolic cages for urine collection. Left: urine was collected for 2.5 h. Right: on a different day (same set of mice), after 30 min, benzamil (following vehicle or insulin plus dextrose) was administered and urine collection continued for 2 more h for a total of 2.5 h. B: in a different set of mice, the same experiment was performed except benzamil was administered 30 min before the insulin or vehicle. Insulin produced antinatriuresis. There was no difference in urinary sodium excretion between the 2 groups after benzamil administration. Furthermore, the difference was completely abolished when the benzamil was injected before insulin or vehicle injections.

*Mean is significantly (P < 0.05) different from that of the vehicle by unpaired t-test.

Fig. 2. Purity of membrane (MF) and vesicle-enriched (VF) fractions as assessed by immunoblotting using antibodies against Na-K-ATPase (membrane-specific marker), GAPDH (cytosolic-specific marker), and syntaxin-4 (apical membrane marker for renal collecting duct cells). For immunoblotting, an equal amount of total protein was loaded from MF and VF from same mouse sample on the same gel (confirmed a priori by equal Coomassie staining of representative protein bands).
membrane-enriched fraction (Fig. 2), an apical membrane-associated protein expressed in renal collecting duct cells.

Increased α-ENaC abundance in the plasma membrane with insulin. Insulin-plus-dextrose-treated mice had significantly increased mean band densities for the whole homogenate (H) and the plasma membrane-enriched fraction (MF) for α-ENaC, relative to vehicle-treated mice (Fig. 3). However, band densities were not significantly different for the vesicle-enriched fraction (VF).

Increased plasma membrane and reduced cytosolic abundance of β-ENaC with insulin. β-ENaC protein abundance was significantly increased in the plasma membrane-enriched fraction (MF) and significantly decreased in the vesicle-enriched fraction (VF) fraction in insulin-plus-dextrose-treated mice relative to vehicle (Fig. 4, A and B). However, no significant difference was observed in the whole homogenate (H) between the two groups.

Increased γ-ENaC abundance in the plasma membrane with insulin. Similar to α-ENaC, γ-ENaC (85-kDa band) was significantly increased in the whole homogenate (H) and plasma membrane (MF) with insulin-plus-dextrose (Fig. 5, A and B). No difference was observed in the VF between the two groups; however, a strong trend for decrease was observed in the insulin-plus-dextrose group. The lower 70- to 80-kDa band associated with γ-ENaC was not significantly affected by treatments and only apparent in the H and MF. Band densities for the vehicle and insulin-plus-dextrose treatment groups for the 70- to 80-kDa band in MF were 100 ± 8.7 (insulin-plus-dextrose) and 108 ± 8.7 (insulin-plus-dextrose), P = 0.7. For the H, the band densities were 100 ± 11.3 (vehicle) and 125 ± 15.2 (insulin-plus-dextrose), P = 0.2.

Increased apical membrane localization of α-, β-, and γ-ENaC with insulin. Immunoperoxidase-based labeling revealed strong apical plasma membrane localization of all three subunits of ENaC in the collecting duct principal cells in insulin-plus-dextrose-treated mice relative to vehicle (Fig. 6A). Moreover, the labeling for β-ENaC was relatively diffuse and somewhat stronger throughout cells in the vehicle-treated mice relative to insulin-plus-dextrose-treated mice. However, the overall labeling for α- and γ-ENaC was stronger in insulin-plus-dextrose-treated mice, relative to vehicle. The semiquantitative analysis using luminosity scoring revealed a significant increase in the staining intensity for ENaC subunits in the apical plasma membrane relative to cytoplasm (Fig. 6B).

Insulin increases plasma membrane SGK-1 and p-S GK-1 abundance. Insulin-plus-dextrose increased SGK-1 band density (~48 kDa) in the MF but not in the H (Fig. 7, A and B). In addition, in the insulin-plus-dextrose-treated mice only, we observed a strong band above the SGK-1 band (~60 kDa), in the MF that has been reported to represent phosphorylated SGK-1 (34). In confirmation, using a p-S GK-1-specific antibody, we found a significant increase in band density with insulin-plus-dextrose (Fig. 7, C and D).
DISCUSSION

Hyperinsulinemia, associated with insulin resistance, is an increasingly common human condition with rising rates of obesity and prediabetes worldwide. Insulin may have a role in sodium retention at the level of the kidney via activation of sodium reabsorption at multiple renal tubule sites. Insulin has been demonstrated to produce antinatriuresis and increase sodium reabsorption in the proximal tubule, the thick ascending limb, and the collecting duct. These nephron segments express a variety of sodium transport-related proteins (17, 45, 49) some of which have already been shown, at the level of cell culture, to be activated by insulin, including the ENaC, the Na-K-ATPase pump, and the sodium phosphate cotransporter (NaPi-2).

Our studies demonstrate, in vivo, a major role for ENaC, a collecting duct principal cell channel, in the antinatriuresis produced by insulin. Benzamil, a specific antagonist of ENaC when given to the mice following insulin-plus-dextrose or vehicle treatments, was able to block 70% of the antinatriuresis due to insulin in these normal mice, so that sodium excreted in the 2-h period was no longer significantly different from vehicle (Fig. 1A). We speculate that the remaining 30% (unblockable by benzamil) could be due to ENaC’s activation by insulin in the first 30 min before it has been blocked by benzamil. To inhibit ENaC’s activation before the insulin treatment, benzamil was administered to the mice 30 min before the treatments (insulin or vehicle). A complete blockage of the antinatriuresis was observed in insulin-plus-dextrose group (Fig. 1B). These results suggest that the antinatriuresis caused by insulin is primarily due to the activation of ENaC. Several lines of evidence suggest that insulin causes antinatriuresis in humans and rats (4, 11, 21, 25, 32). Our result is in agreement with in vitro studies where investigators have used techniques such as electrophysiology to demonstrate the up-regulation ENaC by insulin stimulation (1, 7, 8, 55).

In the same 1- to 2-h time frame, we also demonstrated that mice treated with insulin have increased whole cell abundances of two out of three of the ENaC subunits (α- and γ-ENaC) as determined by band densities on Western blots. This is a short time for expected changes in protein abundance. The observed increased abundance could be either due to increased protein synthesis or decreased degradation of these subunits. In this regard, studies using mammalian kidney and A6 cell lines have demonstrated a fairly brief half-life for ENaC subunits (~1–2 h) in the total cellular pool, and ~1 h at the cell surface (2, 23, 48). Furthermore, in this study, insulin did not lead to any significant enhanced putative cleavage of the (85 kDa) band associated with γ-ENaC. There was no change in band density for the 70- to 80-kDa band region as has been observed with aldosterone stimulation (31) or during vasopressin escape (50).

The increase in the abundance of the α- and γ-subunits in response to insulin but not β could be due to differential trafficking of ENaC subunits.

Fig. 6. Effect of acute insulin on ENaC subunits redistribution in the cortical collecting duct principal cell of the kidney by immunoperoxidase labeling. A: strong apical localization of all the three ENaC subunits was observed in the kidney sections obtained from insulin-injected mice relative to vehicle. B: mean intensity value of ENaC labeling in the collecting duct principal cell was obtained for apical membrane and cytoplasm using Adobe Photoshop-6. Bar graph represents the ratio of the apical membrane to cytoplasmic staining. There was a significant increase in the ENaC subunit staining in the plasma membrane relative to cytoplasm in the insulin-administered group ($n = 5$/group). *Mean is significantly different ($P < 0.05$) from that of the vehicle by unpaired $t$-test.
regulation of subunit abundances by insulin. Selective ERK-mediated phosphorylation of COOH termini of β- and γ- but not of α-subunits, as demonstrated by others (42, 43), is another example of differential regulation of ENaC subunits by insulin. Furthermore, in vivo selective ubiquitination on the α- and γ- (but not β) subunits and their degradation by Nedd4–2, an E3 ubiquitin-ligase, has already been shown (27, 46–48). This would imply noncoordinated regulation of the individual subunits, which is not novel and has been demonstrated in several studies (37, 53, 54).

Thus it is possible that insulin impedes the degradation of α- and γ-ENaC allowing for a greater amount of protein to remain in the plasma membrane and thus an increase in abundance in both the whole homogenate and in the plasma membrane-enriched fractions, as we observed. Our results might also imply that β-ENaC is, in general, of higher abundance. Insulin did not affect its abundance in the whole homogenate, but it did cause a significant reduction in the vesicle-enriched fraction, with a concomitant significant increase in the plasma membrane-enriched fraction (MF). Does this imply reduced synthesis of β-ENaC with insulin or increased trafficking of the existing subunit with insulin? This is clearly not known and will require additional studies.

Interestingly, we (45) found rats treated for 1 mo with insulin infusion did not have any increase in any of the subunits. However, they in agreement with this study did appear to have increased apical localization of α-, β-, and γ-ENaC in the collecting duct principal cells. Insulin has been shown to induce trafficking of ENaC subunits in A6 cells (7) and at the same time also stimulate the changes in lipid composition of plasma membrane (9). We have demonstrated, thus in native tissue, that this redistribution is quite rapid.

Unlike α- and γ-ENaC, we believe that the existing pool of β-ENaC subunit traffics to the apical plasma membrane in response to insulin as suggested by its increase abundance in plasma membrane fraction without any significant change in whole homogenate and a concomitant decrease in vesicle-enriched fraction. This was also quite apparent with immunoperoxidase labeling as well which demonstrated stronger apical relative to cystolic labeling with insulin. In this regard, Weisz et al. (54) showed preferential trafficking of the β-subunit to the apical plasma membrane in response to overnight stimulation with aldosterone or short-term vasopressin stimulation. However, they did not find any change in surface ENaC subunit levels by short-term insulin treatment. The reason for this discrepancy is not clear and could be due to difference in the cell types and dose of insulin used; or due to in vivo vs. in vitro insulin stimulation.

SGK-1 plays an important role in the hormonal regulation of ENaC (1, 3, 52). We found increased SGK-1 in the MF of the kidneys from insulin-treated mice. It is possible, although we have not tested it here, that insulin causes a physical association between ENaC subunits and SGK-1. In this regard, Wang et al. (52) showed a physical association of α- and β-ENaC with both activated (p-SGK) and nonactivated forms of SGK-1 in A6 cells. However, this interaction has not yet been examined in native tissues. It is possible and has been suggested by others as well, that this association may lead to phosphorylation of ENaC directly (52, 55) or activation of ENaC via phosphorylation-dependent inhibition of Nedd4–2 (27, 36).

Finally, it has been shown in cell culture that SGK-1 is a PI3K-dependent inhibitor of insulin-mediated ENaC regulation (52). In agreement with this, we found significant phosphorylation of SGK-1 in the kidney MF from insulin-treated mice.

Overall, we demonstrated acute regulation of ENaC subunit abundances and subcellular distribution by insulin in native tissues from mice treated in vivo with insulin. These changes
correlate temporally with physiological changes in urinary sodium excretion induced by insulin implying a fairly robust role for insulin in the regulation of final urine sodium excretion via these changes in ENaC. We also demonstrate increased SGK-1 and p-S GK-1 in the membrane fraction of kidney from insulin-treated mice in this time frame, suggesting that changes in SGK-1 subcellular distribution and phosphorylation play a role in mechanisms underlying ENaC regulation by insulin in vivo.

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