Effects of insulin on Na and K transporters in the rat CCD

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Frindt G, Palmer LG. Effects of insulin on Na and K transporters in the rat CCD. Am J Physiol Renal Physiol 302: F1227–F1233, 2012. First published February 22, 2012; doi:10.1152/ajprenal.00675.2011.—We tested the effects of insulin (2 nM, 30–60 min) on principal cells of isolated split-open rat cortical collecting duct (CCD) using whole-cell current measurements. Insulin addition to the superfuse of the tubules enhanced Na pump (ouabain-sensitive) current from 18 ± 3 to 31 ± 3 pA/cell in control and from 74 ± 9 to 126 ± 11 pA/cell in high K-fed animals. It also more than doubled ROMK (tertiapin-Q-sensitive) K currents in control CCD from 320 ± 40 to 700 ± 80 pA/cell, although it did not affect this current in tubules from K-loaded rats. Insulin did not induce the appearance of amiloride-sensitive Na current in control animals, while in high K-fed animals the currents were similar in the presence (140 ± 30) and the absence (180 ± 70 pA/cell) of insulin. Intraperitoneal injection of insulin plus hypertonic dextrose decreased Na excretion, as previously reported. However, injection of dextrose alone, or the nonmetabolized sugar mannose, had similar effects, suggesting that they were largely the result of vascular volume depletion rather than specific actions of the hormone. In summary, we find no evidence for acute upregulation of the epithelial Na channel (ENaC) by physiological concentrations of insulin in the mammalian CCD. However, the hormone does activate both the Na/K pump and apical K channels and could, under some conditions, enhance renal K secretion.

ENaC; ROMK; Kir1.1; Na-K-ATPase

THE SYSTEMS THAT CONTROL THE EXCRETION OF K+ IN THE URINE HAVE NOT BEEN FULLY IDENTIFIED. ALTHOUGH ALDOSTERONE HAS BEEN INVOLVED IN THE INCREASED SECRETION OF K+ UNDER CONDITIONS OF HIGH K+ INTAKE (25), EVEN ADRENALECTOMIZED ANIMALS CAN ADAPT TO A DIETARY K+ LOAD (26), IMPLICATING ADDITIONAL KALIURETIC FACTORS. INSULIN IS A CANDIDATE FOR ONE SUCH FACTOR, AS THIS HORMONE HELPS TO CONTROL PLASMA K+ LEVELS CAUSED BY THE HORMONE. RECENTLY, HOWEVER, EVIDENCE HAS BEEN PRESENTED SUPPORTING INCREASED Na REABSORPTION THROUGH THE EPITHELIAL Na CHANNEL (ENaC) IN RESPONSE TO INSULIN (32, 33).

We have directly examined the actions of insulin on apical Na+ and K+ channels as well as on the Na/K pump. We also reevaluated the effects of hormone injection in vivo using the rat as a model system. We conclude that insulin can upregulate ROMK channels and the Na/K pump but find no evidence for an acute effect on ENaC. Stimulation of the K+-transporting system could help to excrete a K+ load after a meal.

METHODS

Animals. All procedures using animals were approved by the Institutional Animal Care and Use Committee of Weill-Cornell Medical College. Sprague-Dawley rats (180–220 g) of either gender (Charles River Laboratories, Kingston, NY) raised free of viral infections were used for all experiments. Animals were fed either normal lab chow or a synthetic diet containing 10% KCl (Harlan Teklad) for 1 wk.

Electrophysiology. Measurement of whole-cell Na+ currents (INa) in principal cells of the CCD followed procedures described previously (11, 12). Split-open tubules were superfused at a rate of ~5 ml/min (mean velocity ~4 mm/s) with solutions prewarmed to 37°C containing (in mM) 135 Na methanesulfonate, 5 KCl, 1 Ca methanesulfonate, 1 MgCl2, 2 glucose, 5 Ba methanesulfonate and 10 HEPES adjusted to pH 7.4 with NaOH. Patch-clamp pipettes were filled with solutions containing (in mM) 7 KCl, 123 aspartic acid, 20 CsOH, 2 TEAOH, 5 EGTA, 10 HEPES, 3 MgATP, and 0.3 NaGDPβS, with the pH adjusted to 7.4 with KOH. The total concentration of K+ was ~120 mM. Net Na+ fluxes through Na+ channels were measured as the difference in current with and without ~10 mM amiloride (Sigma-Aldrich) in the bath. For measurements of whole-cell K+ currents, Ba2+ was omitted from the superfusate. The pipette solutions contained (in mM) 7 KCl, 123 aspartic acid, 5 EGTA, and 10 HEPES, with the pH adjusted to 7.4 with KOH. The total concentration of K+ was ~140 mM. Net K+ fluxes through ROMK channels were measured as the difference in current with and without ~10 mM tertiaipin-Q (TPNQ; Sigma-Aldrich) in the bath.

For measurements of whole-cell Na/K-pump currents, the superfusate was the same as for the measurement of INa. The pipette solutions contained (in mM) 7 KCl, 123 aspartic acid, 20 CsOH, 20 TEAOH, 5 EGTA, 10 HEPES, 3 MgATP and 50 mM NaOH, with the pH adjusted to 7.4 with KOH. The total concentration of K+ was ~70 mM. Pump currents were measured as the difference in current with and without ~10 mM ouabain (Sigma-Aldrich) in the bath.
Pipettes were pulled from hematocrit tubing, coated with Sylgard, and fire polished with a microforge. Pipette resistances ranged from 2 to 5 MΩ. Currents were measured with a List EPC-7 amplifier (Heka Elektronik, Lambrecht, Germany). Voltages were controlled and currents recorded using Pulse software (Heka) and an Instutech ITC-16 interface (Instutech, Mineola, NY).

**Insulin injection.** To assess the effects of insulin in vivo, rats were placed individually in metabolic cages and urine was collected for two intervals of 2 h each. Animals were injected intraperitoneally (ip) with 0.5 U/kg body wt of human insulin (Humulin R) solution with 50% dextrose (2.5 g/kg body wt). Controls received 1 ml sterile H2O ip. In some cases insulin was injected with 1 ml of 5% dextrose. Other animals were given the same dose of hypertonic dextrose alone or an equivalent dose of mannose. After the final urine collection, a sample of blood was obtained from the aorta under anesthesia.

**Analysis.** Na⁺ and K⁺ concentrations in urine and plasma were measured with a flame photometer (model 943, Instrumentation Laboratories, Lexington, MA). Creatinine in plasma and urine was measured using a commercial kit (Infinity, Fisher Diagnostics, Middletown, VA).

**Modeling.** We used a previously developed mathematical model of Na⁺ and K⁺ transport by the CCD (16). For the basal state, we used an intermediate value of apical Na permeability ($P_{\text{Na}}$) of $0.94 \times 10^{-8}$ cm²/s corresponding to a modest level of stimulation by aldosterone (16). Other membrane parameters assumed were the apical K⁺ conductance (440 nS/mm tubule), basolateral K⁺ conductance (5,800 nS/mm), maximal basolateral Na/K pump activity of 17 nA/mm, and a nonspecific paracellular conductance of 300 nS/mm.

**Statistical analysis.** Statistical significance was evaluated using the two-tailed Student’s $t$-test.

**RESULTS**

Insulin increases the activity of the Na/K-ATPase in many cells including those of the CCD (7, 8). To see whether this could be detected electrically in the split-open tubule preparation, we measured the currents generated by the pump as the ouabain-sensitive component of the whole-cell current-voltage ($I$-$V$) relationship (22). The tubules were first opened and preincubated for 30 min in either control solution or with 2 nM (270 U/ml) insulin. For comparison, plasma insulin levels in rats are ~30 U/ml and increase to 60–100 U/ml with a glucose challenge (1, 19, 24, 25). Thus the dose chosen is...
somewhat higher than what the kidneys would normally experience in vivo.

In most tubules, we assessed pump current by adding ouabain while monitoring membrane current in principal cells of CCDs while clamping the membrane voltage to 0 mV. Typical examples for rats on a normal diet are shown in Fig. 1A. In some cases, we measured I-V relationships before and after ouabain administration. Figure 1B shows typical examples of these I-V curves with and without insulin. As previously reported (22), the ouabain-sensitive currents were outward at voltages greater than or equal to −60 mV and tended to saturate at positive cell potentials. Thus currents measured at 0 mV are close to maximal under these conditions. Although using separate tubules for the measurements with and without hormone introduced some variability, the pump currents were consistently larger in the insulin-treated cells, with an average increase of 70% (Fig. 1C). There was no systematic effect of insulin on the extrapolated zero-current voltage or on the current in the presence of ouabain. We also tested effects of insulin on CCDs from rats adapted to a high-K diet. As found earlier (22), this adaptation process entailed a large increase in pump activity. Insulin further increased the pump currents by a factor of 1.7-fold, similar to cells from animals fed the control diet. Thus the effects of high K intake in vivo and insulin in vitro appeared to be independent.

We next examined the effects of the hormone on the function of the renal secretory K⁺ channel Kir1.1 (ROMK). Activity of these channels was assessed as the outward whole-cell current inhibited by the modified honey bee venom peptide TPNQ (Iₛₖ) (14). Although this peptide can under some circumstances also block currents through BK (Maxi-K) channels (17), these channels will not be active under these conditions due to the high concentration of EGTA in the pipette solution, leading to very low levels of cytoplasmic Ca²⁺ (23). Typical tracings are shown in Fig. 2A and B. As with the pump currents, tubules were isolated, split open, and preincubated for 30 min with or without 2 nM insulin. In tubules from control animals, insulin increased Iₛₖ by about twofold (P = 0.0002) (Fig. 2C). Although there was again some variability in the magnitude of these currents from animal to animal, they were larger in the insulin-treated tubules in each of seven rats tested. When the mean values of Iₛₖ from individual rats were compared, the ratio of values from insulin-treated/controls was 2.42 ± 0.44 (P = 0.017). In tubules from animals fed a high-K diet, basal K⁺ currents were considerably larger, consistent with previous findings (14). In this case, however, insulin did not further increase Iₛₖ. Therefore in contrast to the actions on the Na/K pump, the effects of insulin and K loading were not additive.

Finally, we tested the ability of insulin to regulate apical Na⁺ channels. Activity of these channels was measured as inward current inhibited by 10⁻⁵ M amiloride. In control animals, these currents were very low and difficult to detect (Fig. 3A), consistent with previous findings (11, 13). Pretreatment with insulin did not induce significant amiloride-sensitive currents (Fig. 3A). Similar negative results were obtained with 5 nM rat IGF1 (data not shown). One caveat is that basal currents under these conditions were very low, so that even a large fractional increase might be difficult to test. In contrast, when animals were fed a high-K diet Na⁺ currents were easily measurable (Fig. 3B). The amiloride-sensitive I-V relationship shows typical Goldman-type inward rectification. The reversal potentials at +40 to +50 mV presumably reflect accumulation of Na⁺ in the cell close to the membrane by diffusion through open channels. There was no significant difference in these currents in cells treated with 2 nM insulin (Fig. 3, B2 and C).

The absence of an effect of insulin on Na⁺ channels, although consistent with previous measurements using isolated-perfused tubules (see DISCUSSION), was surprising in light of a recent report (32) that insulin injection into mice resulted in an acute, substantial fall in Na excretion that could be reversed with amiloride. We therefore repeated some of these protocols in rats. As shown in Fig. 4A, ip injection of insulin together with 1 ml 50% dextrose to prevent hypoglycemia produced a dramatic decrease in Na⁺ excretion compared with H₂O-injected controls that was maintained over 4 h. These results replicate those reported previously for mice (32).

However, several aspects of this response were not as expected for a specific activation of ENaC. First, K⁺ excretion
fell (Fig. 4B), rather than being stimulated as would be predicted from increased Na\(^+\) channel activity. Second, urine volume flow was strongly depressed (Fig. 4C). Finally, the glomerular filtration rate, estimated from creatinine clearance, decreased by \(\sim30\%\) in the insulin+dextrose-treated animals (Fig. 4D). We also noted in many cases the accumulation of fluid in the peritoneal cavity, suggesting that these effects might result in part from a reduction in extracellular fluids due to the presence of hyperosmotic dextrose.

To test this idea further, we injected dextrose alone, without added insulin. Urine volume and Na\(^+\) excretion fell, similar to those in the insulin+dextrose-treated animals (Fig. 5A), showing that insulin administration was not necessary to evoke the natriferic response. However, it was possible that the sugar elicited the secretion of endogenous insulin secretion. To test this, we injected four animals with insulin plus isotonic dextrose. This concentration of sugar prevented hypoglycemia, but Na\(^+\) excretion and urine volume did not decline under these conditions (data not shown), implying that insulin was not sufficient for the observed anti-natriuresis. Finally, we used 50% mannose instead of glucose. Even in the absence of added insulin, administration of mannose strongly decreased Na\(^+\) excretion (Fig. 5B). Mannose treatment decreased urine volume flow less than did dextrose, probably due to an osmotic diuresis due to urinary excretion of the nonreabsorbable sugar. These results suggest that the reduction in Na\(^+\) excretion observed here and previously is at least in part an indirect effect of the presence of hypertonic fluid in the peritoneal cavity.

DISCUSSION

Effects of insulin on Na-K-ATPase. The stimulation of Na\(^+\) pump activity by insulin that we observed is consistent with previous reports showing that the hormone increased ouabain-sensitive Rb\(^-\) uptake in isolated rat CCDs (7, 8). In those studies, a 30–50\% stimulation was observed after 15-min incubation with 10 nM insulin. The effect was independent of the Na\(^+\) load on the cells. This activation is part of a general pattern of regulation of the pump by the hormone in tissues including skeletal muscle (4, 36), liver (6), adipocytes (20), and renal proximal tubules (7). The mechanism by which this stimulation occurs remains to be established. In adipocytes, the effect appears to involve an increased affinity of the enzyme for intracellular Na\(^+\) (20). However, in the CCD the hormone
did not change the Na⁺ affinity for ATP hydrolysis, indicating an increased maximal turnover rate (8). Our data do not address this issue.

Effects of insulin on ROMK. To our knowledge, this is the first report on the effects of insulin on ROMK channels, the major apical membrane pathway for K⁺ secretion by the distal nephron under most conditions. The increase in ROMK conductance, assessed as TPNQ-sensitive whole-cell current, contrasts with modest inhibition of net K⁺ secretion observed in rabbit CCDs (15). However, in that study Na⁺ transport was also somewhat reduced and the transepithelial voltage was depolarized, indicating that the driving force for K⁺ secretion was diminished. Thus effects on apical K⁺ conductance cannot be inferred from these data.

Effects of insulin on ENaC. The effects of insulin in vitro on epithelial Na⁺ channels appear to be tissue dependent. In amphibian model systems, insulin clearly stimulates ENaC-mediated Na⁺ transport as shown in studies of frog skin (28), toad urinary bladder (3, 29), and A6 cells (2, 9). As discussed above, the rat CCD responds to insulin with an increase in Na-K-ATPase activity. However, in two studies of the isolated, perfused rabbit CCD, the hormone either modestly decreased (15) or did not affect (34) Na⁺ transport. Our failure to observe an effect of insulin on Na⁺ conductance in the rat CCD is consistent with these reports. However, the negative results should be interpreted with caution as we also saw no effect of IGF1 on whole-cell ENaC currents under these conditions, while a previous study found stimulation of Na channels by this hormone in cell-attached patches of rat CCD (31). We do not know the basis for this discrepancy.

Effects of insulin in vivo. Several whole-kidney and in vivo studies suggested that insulin is antinatriuretic, although the site of action of the hormone was not clearly identified. Nizet et al. (21) found that insulin (80–300 μU/ml) reduced Na⁺, K⁺, and H₂O excretion in perfused dog kidneys without any changes in glomerular filtration rate, plasma glucose, or K⁺. They suggested that the increase in Na⁺ reabsorption caused by insulin took place at a nephron segment different from that where Na⁺ is exchanged for K⁺. DeFronzo et al. (5) infused insulin, together with glucose to maintain euglycemia, into human subjects and reported decreases in Na⁺ and K⁺ excretion, while plasma K⁺ fell from 3.8 to 3.1 mM. They suggested that the increased Na⁺ reabsorption occurred in the diluting segment or more distally in the renal tubules, while decreased plasma K⁺ lowered K⁺ secretion. Kirchner (18) studied glucose-clamped, insulin-infused rats using free-flow micropuncture. He found that plasma insulin (up to 46 μU/ml) reduced Na⁺, Cl⁻, and water excretion. Insulin markedly increased Cl⁻ reabsorption in the loop of Henle, but it had no effect on Cl⁻ delivery out of the proximal convoluted tubule or on distal tubule fractional Cl⁻ reabsorption.

Other investigations, however, failed to show direct insulin-induced increases in Na⁺ reabsorption. In humans maintained euglycemic, Friedberg et al. (10) found that insulin decreased Na⁺ and K⁺ excretion but that this effect was abrogated when K⁺ was infused with insulin to maintain plasma K⁺.
et al. (10) infused insulin directly into one dog renal artery. Fractional excretions of Na\(^+\)/H\(^+\) and K\(^+\)/H\(^+\) were not different from those in the contralateral renal artery infused with vehicle. Rosseti et al. (27) assessed the effects of insulin on renal electrolyte excretion in saline-infused rats in which both plasma glucose and plasma K\(^+\)/H\(^+\) levels could be controlled. In euglycemic animals, elevation of plasma insulin levels to 380\(/\mu\text{g/ml}\) without administration of K\(^+\)/H\(^+\) caused a fall in plasma K\(^+\) of 0.35 mM and no change in K\(^+\)/H\(^+\) excretion. In animals infused with K\(^+\)/H\(^+\) to maintain constant plasma levels, insulin increased K\(^+\)/H\(^+\) excretion by 2.5-fold but did not affect Na\(^+\)/H\(^+\) excretion. Our results are in general accordance with these findings.

Most recently, Tiwari et al. (32) observed a strong decrease in Na\(^+\)/H\(^+\) excretion of mice treated with insulin plus hyperosmotic dextrose to maintain euglycemia. As discussed above, we believe that this effect is due at least in part to the hyperosmotic fluid added to the peritoneal cavity. Although we cannot rule out a natriferic response to insulin, we think it will be necessary to reevaluate this model, avoiding possible changes in extracellular volume and osmolarity associated with the dextrose administration.

**Regulation of K\(^+\) excretion.** Although we could not document an effect of insulin on Na\(^+\) channels, believed to be the rate-limiting step in transepithelial Na\(^+\) transport, we did observe increases in the activities of transporters directly involved in K\(^+\) secretion, namely apical K\(^+\) (ROMK) channels and the Na-K-ATPase. To evaluate the impact of these changes on K\(^+\) secretion, we used a numerical model of Na\(^+\) and K\(^+\) transport developed previously (14, 16). Figure 6 shows the results of the analysis. Baseline values were chosen to mimic rates of Na\(^+\) and K\(^+\) transport observed under conditions of a modest level of ENaC activity (16). A twofold increase in apical K\(^+\) conductance (G\(_{\text{Ka}}\)), similar to what we observed for rats on a normal rodent diet, increased net K\(^+\) secretion rates by 40–60%, depending on apical P\(_{\text{Na}}\). Increasing the pump activity by twofold induced smaller rises, from 1 to 10%, in K\(^+\) transport. Generally, the two effects of the two perturbations were additive. However, at the highest P\(_{\text{Na}}\), increasing G\(_{\text{Ka}}\) and basolateral pump density were synergistic. This was the result of a large rise in intracellular Na\(^+\) from 19 to 48 mM with increased G\(_{\text{Ka}}\), saturating the activity of the pump when its maximal turnover rate was limiting.

Overall, these calculations suggest that the observed actions of insulin could help to explain the findings of Rossetti et al. (27) discussed above. Administration of insulin to saline-loaded rats that were kept both euglycemic and eukalemic resulted in increased K\(^+\) excretion with little change in Na\(^+\) excretion. Although the calculated Na\(^+\) reabsorption through ENaC channels increases as a result of apical membrane hyperpolarization when K\(^+\) channels are activated, this effect is relatively small and might be compensated by other Na\(^+\) transporters.

Such a mechanism provides a means of excreting excess K\(^+\) after absorption of a meal, and the expected secretion of insulin. The hormone would be even more effective in this role if it also provoked a concomitant increase in Na\(^+\) channel...
activity, but we could not document such a stimulation. The accelerated K⁺ secretion would act together with increased uptake of K⁺ into cells in maintaining plasma K⁺ within normal limits.

GRANTS
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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS
Author contributions: G.F. and L.G.P. provided conception and design of research; G.F. and L.G.P. performed experiments; G.F. and L.G.P. analyzed data; G.F. and L.G.P. interpreted results of experiments; G.F. and L.G.P. edited and revised manuscript; G.F. and L.G.P. approved final version of manuscript; L.G.P. prepared figures; L.G.P. drafted manuscript.

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