Decreased renal perfusion rapidly increases plasma membrane Na-K-ATPase in rat cortex by an angiotensin II-dependent mechanism

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1Department of Physiology, 2Division of Pulmonary, Allergy, Critical Care and Sleep Medicine, Department of Internal Medicine, and 3Department of Pharmacology, Wayne State University School of Medicine, Detroit; and 4Division of Hypertension and Vascular Research, Henry Ford Hospital, Detroit, Michigan

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Yingst DR, Araghi A, Doci TM, Mattingly R, Beierwaltes WH. Decreased renal perfusion rapidly increases plasma membrane Na-K-ATPase in rat cortex by an angiotensin II-dependent mechanism. Am J Physiol Renal Physiol 297: F1324–F1329, 2009. First published September 2, 2009; doi:10.1152/ajprenal.90363.2008.—To understand how rapid changes in blood pressure can regulate Na-K-ATPase in the kidney cortex, we tested the hypothesis that a short-term (5 min) decrease in renal perfusion pressure will increase the amount of Na-K-ATPase in the plasma membranes by an angiotensin II-dependent mechanism. The abdominal aorta of anesthetized Sprague-Dawley rats was constricted with a ligature between the renal arteries, and pressure was monitored on either side during acute constriction. Left renal perfusion pressure was reduced to 70 ± 1 mmHg (n = 6), whereas right renal perfusion pressure was 112 ± 4 mmHg. In control (nonconstricted) rats (n = 5), pressure to both kidneys was similar at 119 ± 6 mmHg. After 5 min of reduced perfusion, femoral venous samples were taken for plasma renin activity (PRA) and the kidneys excised. The cortex was dissected, minced, sieved, and biotinylated. Lower perfusion left kidneys showed a 41% increase (P < 0.003) in the amount of Na-K-ATPase in the plasma membrane compared with right kidneys. In controls, there was no difference in cell surface Na-K-ATPase between left and right kidneys (P = 0.47). PRA was 57% higher in experimental animals compared with controls. To test the role of angiotensin II in mediating the increase in Na-K-ATPase, we repeated the experiments (n = 6) in rats treated with ramiprilat. When angiotensin-converting enzyme was inhibited, the cell surface Na-K-ATPase of the two kidneys was equal (P = 0.46). These results confirm our hypothesis: rapid changes in blood pressure regulate trafficking of Na-K-ATPase in the kidney cortex.

Conversely, an acute increase in blood pressure reversibly inhibits Na-K-ATPase activity in the rat proximal tubule (23). This result suggests there could be a dynamic interaction between Na-K-ATPase activity and blood pressure, which is of physiological importance. If this is the case, then a rapid drop in blood pressure would be expected to stimulate Na-K-ATPase activity. Therefore, in this study we have tested the hypothesis that in vivo, a short-term (5 min) decrease in renal perfusion pressure will increase the amount of Na-K-ATPase in the plasma membranes of the kidney cortex by a dynamic interaction between blood pressure and the production of ANG II.

Our experimental approach was to reduce the perfusion pressure to one kidney while maintaining the other contralateral kidney at a normal perfusion pressure. Our expectation was that reducing the perfusion pressure to one kidney would result in an increase in the amount of Na-K-ATPase in the plasma membranes of that kidney compared with the contralateral kidney without any change in the total amount of Na-K-ATPase in the cell. There should be no change in the total amount of Na-K-ATPase in the cell because the period of low pressure (5 min) is too short for there to be an increase in protein expression. We expected that the decrease in perfusion pressure would likely lead to an increase in the plasma concentrations of ANG II but also, more importantly, to a selective increase in the amount of ANG II in the low-pressure kidney compared with the contralateral kidney, as has been observed over a longer period of time (days) in a model of renovascular hypertension (19). On the basis of results in cultured cells (5), noted above, we expected this increase in ANG II would lead to an accumulation of cellular Na-K-ATPase in the plasma membrane. We anticipated that there would be an increase in the amount of Na-K-ATPase in the plasma membrane of the low-pressure kidney compared with the contralateral kidney, if there was indeed a selective increase in the amount of intrarenal ANG II in the low-pressure kidney. We expected there to be a relationship between blood pressure and the production of ANG II, because renin secretion, the rate-limiting step in the formation of angiotensin, is increased through the renal baroreceptor mechanism (20) when renal perfusion pressure is lowered near the lower limit of renal blood flow autoregulation (3). Finally, we anticipated that these studies would support the concept (23) that there is a dynamic interactive relationship between rapid changes in blood pressure and Na-K-ATPase activity in the kidney cortex.

METHODS

Animal Preparation

Male Sprague-Dawley rats (Charles River Laboratory, Wilmington, MA) were anesthetized using thiobutabarbital (Inactin; 125 mg/kg body wt ip) and placed on a heating pad to maintain body temperature. A tracheostomy was performed using polyethylene (PE)-
240 tubing to allow spontaneous breathing of room air. The femoral vein was catheterized with PE-50 tubing for maintenance infusion of 40 μL/min of 0.9% NaCl and for collection of venous blood. Femoral venous blood was sampled slowly in 250-μL volumes and then replaced with an equal amount of 6% heat-inactivated BSA. Both the left carotid artery and the right femoral artery were catheterized using heparinized PE-50 tubing attached to a three-way stopcock on a Statham pressure transducer (Viggo-Spectramed, Oxnard, CA; calibrated using a mercury manometer) and a Gould recorder (Gould Instruments, Valley View, OH) for continuous monitoring of supra-renal systemic blood pressure (BP) and renal perfusion pressure, respectively. The abdominal cavity was opened via a midventral incision. The intestines were wrapped in warm, moist gauze and tucked under the right ventral wall, and both the left and right renal arteries and veins were dissected from the surrounding tissues. To control renal perfusion pressure to the left kidney, a 1.0 silk ligature was placed around the aorta between the origins of the two renal arteries and then threaded through a plastic sleeve made from PE-140 tubing to allow spontaneous breathing of room air. The femoral artery catheter, to which the aortic constrictor was then tightened to reduce the renal perfusion pressure to only the left kidney, and left renal perfusion pressure was monitored via the femoral artery catheter while systemic perfusion of the contralateral right kidney was maintained. Control rats had an identical surgical preparation, but the ligature was not constricted, leaving both kidneys perfused with the same pressure. After surgery, the rats received a supplemental bolus of 1.0 mL of 6% heat-inactivated BSA in normal saline and stabilized for 45–60 min. At the conclusion of the protocol, rats were killed by bilateral nephrectomy and pneumothorax while still under anesthesia. All procedures using animals were approved by our Institutional Animal Care and Use Committee and adhere to the guiding principles in the care and use of experimental animals. Henry Ford Hospital’s animal facility is approved by the American Association for the Accreditation of Laboratory Animal Care.

**Experimental Protocols**

Rats were treated using one of three different experimental protocols.

**Group 1.** Rats in group 1 (n = 6) were subjected to different renal perfusion pressures (ΔP) in the right and left kidney. To do this, once the rat had stabilized 45–60 min after surgery, systemic pressure was noted, and a blood sample for plasma renin activity (PRA) was obtained from the femoral venous catheter. The aortic ligature between the renal arteries was then tightened to reduce the renal perfusion pressure to the left kidney, as monitored through the femoral arterial catheter, to ~70 mmHg. Once stabilized, this pressure was maintained for 5 min. At the conclusion of this period, the pressure to the right kidney was also determined via the carotid catheter, and a second blood sample for PRA was obtained. The left kidney was then removed, decapsulated, longitudinally transected, and placed in iced saline. The right kidney was also immediately removed, decapsulated, longitudinally transected, and placed in iced saline for processing as described below.

**Group 2.** Rats in this group (n = 5) were treated identically with those in group 1, except that the aortic constrictor was not closed to reduce renal perfusion pressure to the left kidney, and both kidneys remained at normal systemic pressure throughout the 5-min experimental period. These animals served as equal pressure controls.

**Group 3.** Rats in this group (n = 6) were allowed to stabilize and were then administered a 50 μg/kg body wt bolus of the ANG II-converting enzyme inhibitor ramiprilat. Rats that responded to the drug with a transient decrease of more than 10 mmHg were excluded to eliminate any errant depressor signals confounding our results. After 60 min, once blood pressure was again stable, the protocol of unilateral reduced renal perfusion pressure identical to that in group 1 was repeated, reducing the renal perfusion pressure to ~70 mmHg over 5 min.

**Tissue Processing**

Immediately after the kidneys were removed from the rat, the cortex of each kidney was dissected, minced separately in iced phosphate-buffered saline (PBS), and then pressed through a 250-μm stainless steel sieve with a spoonula. The tissue slurry was centrifuged at 1,164 relative centrifugal force (RCF) for 10 min at 4°C, and the pellet containing the proximal-rich renal cortical sample was resuspended into a borate buffer (10 mM boric acid, 140 mM NaCl, 4 mM KCl, and 1.8 mM CaCl2; pH 8.5) containing 1.5 mg/mL NHS-biotin dissolved in DMSO.

Biotinylation labeling of the membrane-bound Na-K-ATPase was carried out over a 45-min period divided into 15-min incubations in which the incubation medium was replaced with fresh biotin-containing medium. Samples were placed in capped 50-ml tubes with two small holes in the cap, one for venting and one for the gas line. The tissue was suspended in aerated medium at 4°C and overlaid with a flow of 95% O2-5% CO2 for 15 min. The tube was then centrifuged for 3 min to pellet the tissue, the incubation medium was vacuum aspirated and then replaced with new medium, the pellet was resuspended, and the next 15-min incubation period was begun. The biotinylated tissue was then washed twice with centrifugation with a lysine buffer (100 mM lysine in PBS, pH 7.8) to remove free biotin. Finally, 5 ml of chilled 3 mM ATP in PBS were added to the pellet, and the sample was placed on ice.

Next, the collected samples were vortexed, and a 1-ml aliquot of each sample was quickly removed and placed in an ice-cold microcentrifuge tube. The samples were centrifuged at 16,000 RCF for 10 min at 4°C, and the supernatant was discarded. A volume of 0.5 ml of cold RIPA buffer containing 2% SDS and protease inhibitors (22) was added to the pellet, and the tube was vortexed for 10 min at 4°C. Another 0.25 ml of the same solution was added, and the mixing continued. Ten minutes later, another 0.25 ml of the same mixture was added, and the mixing continued for another 10-min period. The sample was then centrifuged at 16,000 RCF for 10 min at 4°C. The supernatant was removed, and the protein concentration was determined using bicinchoninic acid following the manufacturer’s suggestions. The final protein concentration was ~4 mg/mL. An aliquot containing 0.3 mg of protein was removed, and the volume was increased to 0.5 ml by adding RIPA buffer supplemented with protease inhibitors (22). This sample was combined with 0.5 ml of immobilized streptavidin that had been previously washed twice with ice-cold RIPA buffer. The suspension was incubated overnight at 4°C with gentle end-over-end mixing. The next morning, the samples were briefly centrifuged to pellet the streptavidin, and the supernatant was removed to separate tubes. The streptavidin was then washed first with RIPA buffer and then with a high osmotic buffer containing 500 mM NaCl, 5 mM EDTA, 50 mM Tris, and 0.1% Triton and, finally, with a low osmotic buffer containing 50 mM Tris (8). The isolated membrane proteins were removed from the streptavidin by adding 0.5 ml of Laemmli sample buffer (9) containing excess DTT and β-mercaptoethanol.

The relative amount of biotinylated Na-K-ATPase in the right and left kidneys from each experiment was measured by immunoblotting (15) for the α-subunit of the Na-K-ATPase by using chemiluminescence as previously described (22). On each blot we ran multiple samples from the left and right kidneys from a given experiment along with known amounts of rat kidney microsomes (22). The total amount of protein added per lane was ~5 μg or less. For instance, in each experiment, we took an ~8-μl aliquot containing ~5 μg of protein from the 0.5 ml of sample that was added to the streptavidin. An equal aliquot was then taken at each subsequent step in the procedure. Immunoblot signals were quantified in arbitrary units using a Fuji LAS-1000 System and Image Gauge version 3.3 software. For each blot we then constructed a standard curve of arbitrary units vs. known amounts of rat kidney microsomes and fit the data with the equation \[ y = (a)\ln x + b \] using least squares. This equation was then used for
decreased renal perfusion increases Na-K-ATPase

<table>
<thead>
<tr>
<th>Type of Experiment</th>
<th>Δ P (n=6)</th>
<th>control (n=5)</th>
<th>Δ P + ACEi (n=6)</th>
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<tbody>
<tr>
<td>Kidney</td>
<td>Left</td>
<td>Right</td>
<td>Left</td>
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<tr>
<td>Perfusion Pressure (mm of Hg)</td>
<td>Low Normal</td>
<td>Normal Normal</td>
<td>Low Normal</td>
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<tr>
<td>Biotinylated α-subunit recovered in a typical experiment</td>
<td>(70 ± 4)</td>
<td>(121 ± 6)</td>
<td>(119 ± 6)</td>
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Fig. 1. Perfusion pressure of the left and right kidneys and an example of the amount of biotinylated Na-K-ATPase recovered from left and right kidneys in the 3 types of experiments performed in this study: pressure change (ΔP, group 1), control (group 2), and pressure change in the presence of ANG II-converting enzyme inhibitor (ΔP + ACEi, group 3). Values of perfusion pressures are means ± SE for the number (n) of experiments shown. Immunoblots are shown for the α-subunit of Na-K-ATPase.

The samples on that blot to calculate the amount of rat kidney microsomes (in μg) that would have contained the same amount of Na-K-ATPase as the samples. Because each left kidney was paired with its own contralateral right kidney, we tested for differences in the amount of Na-K-ATPase in the plasma membranes of right and left kidneys using a paired t-test. A P value <0.05 was considered to be significant. Blots that had been probed for anti-α-subunit were stripped and probed for anti-tubulin (14).

For each experiment we also measured the total amount of Na-K-ATPase in the detergent-dissolved samples from the left and right kidneys by using the same immunoblotting procedure and found that there was no significant difference. We also confirmed that there was no Na-K-ATPase coming off the streptavidin before we added reducing agents. Controls were also run to verify that the biotin was not labeling intracellular proteins. These issues are directly addressed in the RESULTS.

PRA Assay

PRA was determined from femoral venous blood samples as previously described (2). PRA was assayed for the generation of angiotensin I using a Gamma Coat RIA kit (DiaSorin, Stillwater, MN). Units are expressed in nanograms of ANG I per hour.

Materials

We purchased acrylamide from Bio-Rad Laboratories (Hercules, CA); PVDF from Millipore (Billerica, MA); BCA, NHS-SS-biotin, and immobilized streptavidin from Pierce Biotechnology (Rockford, IL); goat horseradish peroxidase-conjugated secondary anti-mouse antibodies from Jackson ImmunoResearch (West Grove, PA); KPL chemiluminescence reagents from Insight Biotechnology (Wembley, UK); and ramiprilat from Astra Zeneca (Newark, DE). Mouse anti-tubulin antibody was obtained from the Developmental Studies Hybridoma Bank (Iowa City, IA). All other reagents, including protease inhibitor cocktail and the anti-α-subunit antibody (M8-P1-A3), were purchased from Sigma-Aldrich (St. Louis, MO).

RESULTS

Group 1

We first tested whether a brief reduction in renal perfusion pressure would increase the incorporation of Na-K-ATPase into the plasma membranes of the kidney cortex. In these experiments the perfusion pressure to the left kidney was reduced to 70 ± 1 mmHg, a change of ~40 mmHg from that to the nonconstricted right kidney (Fig. 1). We found more biotinylated Na-K-ATPase recovered from the left, low-pressure kidney than from the right kidney maintained at a normal perfusion pressure (Fig. 1). In these experiments, the amount of protein analyzed by means of immunoblotting was well within the range in which the intensity of the response was sensitive to the amount of Na-K-ATPase present (Fig. 2). The immunoblot signal from biotinylated α-subunit from the left kidneys (mean ± SE) was the equivalent of 0.87 ± 0.20 μg of rat kidney microsomes compared with 0.69 ± 0.20 μg in the right kidneys maintained at normal pressure, which were significantly different from each other (P = 0.003) as shown by a paired t-test.

The amount of biotinylated Na-K-ATPase recovered in these experiments varied between 20 and 40% of the total amount of Na-K-ATPase in the cell (data not shown). The biotinylated Na-K-ATPase that was eluted from the streptavidin is from the plasma membranes of the kidney cortex and not from intracellular vesicles, because intracellular proteins were not eluted from the streptavidin (Fig. 3). For example, both the Na-K-ATPase, which can be in either the plasma membrane or intracellular vesicles, and tubulin, which is only found intracellularly, were present in the mixture of cellular proteins from left and right kidneys that were combined with the streptavidin (Fig. 3, lanes 1 and 8); both were present in the mixture of proteins that did not bind to the streptavidin (Fig. 3, lanes 2 and 7); but only Na-K-ATPase was present in the sample that was released from the streptavidin on the addition of reducing agent (Fig. 3, lanes 4 and 5).
Na-K-ATPase in the plasma membranes of both kidneys is the
same. Likewise, there was no difference between the amount of
Na-K-ATPase in the plasma membranes of the right kidneys in
group 1 compared with group 2 as analyzed using a normal
\( t \)-test (\( P = 0.47 \)).

**Group 3**

In this group with angiotensin-converting enzyme inhibited
(ACEi), reducing renal perfusion pressure to the left kidney
failed to change the amount of Na-K-ATPase in the plasma
membranes from the renal cortex compared with the amount in
the right kidney maintained at the basal renal perfusion pres-
sure (Fig. 1). The \( \alpha \)-subunit in the left, reduced-pressure
kidneys was 0.81 ± 0.22 \( \mu g \) compared with 0.79 ± 0.14 \( \mu g \)
in the right kidneys. These values were not different from each
other (\( P = 0.46 \)) as analyzed using a paired \( t \)-test, and the ratio
of Na-K-ATPase in the left and right kidneys was not different
from 1 (Fig. 4). These data suggest the increase in the incor-
poration of Na-K-ATPase in the plasma membranes of cells in
the cortex of the kidney in response to a decrease in perfusion
pressure is mediated, at least in part, by a local increase in
ANG II. We could detect no difference in the amount of
Na-K-ATPase in the plasma membranes of the right (normal
pressure) kidney of rats in group 1 (\( \Delta P \)) and group 3 (\( \Delta P \+ 
ACEi \)) as determined using a normal \( t \)-test (\( P = 0.35 \)). The
latter result shows that there is no evidence that the adminis-
tration of ramiprilat significantly changed the amount of Na-
K-ATPase in the plasma membrane under our experimental
conditions.

To compensate for differences between experiments, we
also calculated the ratio of biotinylated Na-K-ATPase in the
left renal cortex compared with the right renal cortex in each
experiment. This ratio will most accurately reflect the differ-
ces in biotinylated Na-K-ATPase between the two kidneys.
We found a significant increase in the amount of biotinylated
Na-K-ATPase in the low-pressure kidney of >40% (Fig. 4).
These data suggest that decreasing the perfusion pressure for a
brief 5-min period significantly increased the amount of Na-
K-ATPase in the plasma membranes of cells that were in the
kidney cortex.

To determine whether these acute changes in renal perfusion
pressure would result in stimulating the renin-angiotensin sys-
tem, we measured PRA as an index of angiotensin formation.
PRA increased 57% from 9.7 ± 2.5 to 15.2 ± 2.5 ng ANG
1·ml\(^{-1}\)·h\(^{-1}\) over the 5-min period in which the perfusion
pressure was reduced.

**Group 2**

In these control experiments, the perfusion pressure to both
kidneys was maintained at equal pressures for 5 min (Fig. 1).
The amount of Na-K-ATPase in the plasma membranes of the
left and right kidneys had equal amounts of biotinylated Na-
K-ATPase (Fig. 1). When the amount of \( \alpha \)-subunit was quan-
tified, the values (means ± SE) were 0.68 ± 0.11 \( \mu g \) for the
left kidney and 0.67 ± 0.09 \( \mu g \) for the right kidney, which
were not different from each other (\( P = 0.47 \)) as determined by
a paired \( t \)-test. The mean ratio of biotinylated \( \alpha \)-subunit in the
left kidney compared with the right kidney was not different from 1 (Fig. 4), meaning that the cortices from both the left and
right kidneys contained the same amount of Na-K-ATPase in
their plasma membranes. These data confirm that when the
perfusion pressure is the same in both kidneys, the amount of
Na-K-ATPase in the plasma membranes of both kidneys is the
same.
DISCUSSION

Our results directly support the hypothesis that, in vivo, a short-term (5 min) decrease in renal perfusion pressure will increase the amount of Na-K-ATPase in the plasma membranes of the kidney cortex by an ANG II-dependent mechanism without a change in the total amount of cellular Na-K-ATPase. This rapid increase in the amount of Na-K-ATPase in the plasma membrane in response to a decrease in perfusion pressure supports the concept that there is a dynamic interaction between blood pressure and Na-K-ATPase activity in the kidney cortex. It was previously known that an acute short-term increase in blood pressure rapidly inhibits Na-K-ATPase activity (23). Now we also know that a rapid decrease in pressure will increase the amount of Na-K-ATPase in the plasma membrane, which would be predicted to increase Na-K-ATPase activity. An increase in activity would be expected to initiate an increase in sodium reabsorption, which could help restore blood volume and normalize blood pressure.

Our results also support the conclusion that this rapid increase in the amount of Na-K-ATPase in the plasma membrane is mediated by ANG II. ANG II is implicated because ramiprilat, an ANG II-converting enzyme inhibitor, blocked the increase in the amount of Na-K-ATPase in the plasma membrane of the low-pressure kidney relative to the contralateral kidney maintained at a normal perfusion pressure in group 1. The increased amounts of Na-K-ATPase in the plasma membranes of the low-pressure kidney are likely due to a local increase in intrarenal ANG II in the low-pressure kidney compared with the contralateral kidney. Although it is true that we observed an increase in PRA in the rats in group 1, it is difficult to see how an increase in PRA and circulating ANG II can explain the preferential increase in Na-K-ATPase in the plasma membranes of the low-pressure kidney. There is no evidence that this increase in PRA had an effect on the amount of Na-K-ATPase in the plasma membranes of both kidneys, because we did not detect any increase in the amount of Na-K-ATPase in the plasma membranes of the right kidney in group 1 compared with group 2. Likewise, there was not a significant difference in the absolute amount of Na-K-ATPase that was in the right kidneys in groups 1 and 3 that had been treated with ramiprilat. It is, of course, possible that differences in the amount of biotinylated Na-K-ATPase between experiments obscured our ability to detect a difference in these unpaired comparisons. Nevertheless, it is difficult to see how an increase in the plasma levels of ANG II can explain the increased amounts of Na-K-ATPase in the low-pressure kidney relative to its matched control.

A role for ANG II in regulating the amount of Na-K-ATPase in the plasma membrane in response to a change in blood pressure is consistent with the observation that a drop in plasma ANG II concentration mediates inhibition of sodium reabsorption (11) and a step in the redistribution of Na/H exchanger 3 (NHE3) in the apical membrane of the proximal tubule (10) in response to an acute increase in blood pressure. Zhou and colleagues have suggested that in proximal tubules, ANG II acting through AT1 receptors in either the plasma membrane or internalized vesicles (12) stimulates a series of cellular signaling events including increased intracellular calcium (25), activation of NF-kB (24), redistribution of NHE3, and regulation of proximal sodium transport (13). The involvement of ANG II in mediating Na-K-ATPase in the plasma membrane of proximal tubular cells would complement the proposed ANG II mediation of NHE3 (10, 13) in local regulation of proximal sodium reabsorption.

It has been known for some time that ANG II-induced changes in the phosphorylation of Na-K-ATPase directly stimulate Na-K-ATPase activity in the rat proximal tubule (1, 7) and that this effect could occur in as little as 2 min (21). However, it was first suggested that the stimulation was due to an increase in the affinity of the Na-K-ATPase for intracellular sodium (1), its rate-limiting substrate. It was later shown in opsonum kidney cells that express the rat kidney Na-K-ATPase that ANG II directly stimulates Na-K-ATPase activity in ~15 min or less via the AT1 receptor (5). Activation of the AT1 receptor stimulates PKC, which in turn phosphorylates the rat Na-K-ATPase at Ser11 and Ser18, which triggers a rapid accumulation of Na-K-ATPase in the plasma membrane (5). Our results are the first to show that this trafficking-type mechanism may also be relevant to how ANG II directly stimulates Na-K-ATPase activity in intact animals and to relate this effect to changes in blood pressure.

The concept that the amount of Na-K-ATPase in the plasma membrane quickly (≤5 min) responds to physiological changes of renal perfusion pressure and baroreceptor-mediated angiotensin formation near the lower limit of renal autoregulation (3) may represent an underappreciated mechanism with a fast response to preserve sodium. Although this may reflect the response to rapid changes and normal fluctuations in blood pressure, it also may be part of a complex mechanism involved in renal pathophysiology, such as adaptation of the stenotic kidney in the development of renovascular hypertension (17, 18), or more rigorous compromises to renal function such as the response to hypovolemic shock. Greater reductions in perfusion pressure than what we used in our experiments would be expected to compromise renal blood flow, leading to ATP depletion and associated cellular changes (16). Changes in the trafficking of Na-K-ATPase to the plasma membrane in response to changes in pressure could be a major factor in pathophysiology, such as adaptation of the stenotic kidney in the development of renovascular hypertension (17, 18), or more rigorous compromises to renal function, such as the response to hypovolemic shock. None of these has yet been studied in the context of sodium transport controlled by local ANG II production, nor has the pathway between AT1-mediated signaling and translocation and incorporation of the Na-K-ATPase in the plasma membrane, but these may reveal a new understanding of local, filtered, or reabsorbed ANG II as a key factor in controlling sodium reabsorption. The current study purposefully does not address these more aggressive and complex responses.

In conclusion, we found that acute reductions in renal perfusion pressure in vivo lead to a significant increase in Na-K-ATPase incorporation into the plasma membrane of cells in the renal cortex. Furthermore, we observed that the unilateral renal baroreceptor stimulation of renin, and presumably local tissue generation of ANG II, is the signal that mediates this increased trafficking and incorporation of Na-K-ATPase into the basolateral membrane. This response is consistent with an acute increase in the ability to reabsorb sodium and help maintain blood pressure in the face of a perceived decline in renal perfusion pressure.
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GRANTS

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