Angiotensin II Increases H\(^+\)-ATPase B1 Subunit Expression in Medullary Collecting Ducts

Patricia Valles, Jan Wysocki, Mohammed Reza Salabat, Ivan Cokic, Minghao Ye, Michael S. LaPointe, Daniel Batlle

Abstract—Metabolic alkalosis is a common feature of hypokalemic hypertensive syndromes associated with angiotensin II excess. The alkalosis-generating effect of angiotensin II is usually ascribed to its stimulatory effect on aldosterone secretion, a hormone that upregulates collecting duct hydrogen ion secretion. We studied the effect of angiotensin II infusions on the expression of B1 and a4 protein, subunits of the renal H\(^+\)-ATPase in adrenalectomized rats. Adrenalectomized rats were given either angiotensin II or vehicle for 7 days via osmotic mini-pumps. H\(^+\)-ATPase B1 protein expression was evaluated by Western blot analysis in isolated medulla and cortex plasma membrane preparations from one kidney, whereas the contralateral kidney was used for immunostaining. By Western blotting, the relative abundance of B1 protein was 2-fold higher in renal medulla membranes from rats with intact adrenal glands (sham surgery) than from adrenalectomized rats (219±47%, n=12; P<0.05). In contrast to renal medulla, adrenalectomy did not significantly alter the relative abundance of B1 protein in renal cortex. Angiotensin II also did not significantly alter the relative levels of B1 protein in the cortex, but it increased it significantly in renal medullary membranes (231±56%, n=8; P<0.005). Moreover, enhanced H\(^+\)-ATPase B1 subunit protein immunoreactivity was found in medullary collecting duct segments of rats infused with angiotensin II. In contrast to B1, expression of a4, another subunit of the H\(^+\)-ATPase was not altered by adrenalectomy or angiotensin II. We conclude that adrenalectomy decreases whereas angiotensin II increases H\(^+\)-ATPase B1 subunit expression in medullary, but not in cortical collecting ducts. By increasing the relative abundance of the B1 subunit of H\(^+\)-ATPase in the collecting duct, angiotensin II excess may lead to increased hydrogen ion secretion and thus metabolic alkalosis—a common feature of hypertensive syndromes associated with angiotensin II overactivity. (Hypertension. 2005;45:1-6.)

Key Words: aldosterone ■ angiotensin II ■ mineralocorticoids

The V-H\(^+\)-ATPases are a family of multisubunit ATP-dependent proton pumps responsible for acidification of intracellular organelles and acidification of lumina or interstitial spaces adjacent to cell plasma membranes.1,2 The V-H\(^+\)-ATPase, a heterooligomeric complex composed of 13 polypeptide types, can be fractionated into a soluble cytoplasmically disposed V\(_{t}\), a catalytic domain of 570 kDa that encompasses the ATPase activity, and a membrane-associated V\(_{o}\) domain of 260 kDa that includes the proton translocation pathway.3–5 Kidney-relevant mutations in 2 different subunits of the vacuolar H\(^+\)-ATPase have been described. Mutations in either the B1 or the 116-kDa subunit (a4 isoform) have a distinctive inherited clinical phenotype characterized by the features of distal renal tubular acidosis.6,7 Mutations in the B1 subunit (ATP6B1) cause distal renal tubular acidosis with sensorineural deafness.6 The 116-kDa subunit (ATP6B1) cause distal renal tubular acidosis with sensorineural deafness.6 The 116-kDa subunit (a4) is found in the early segments of proximal tubules and in the intercalated cells in the kidney.8 The B1 subunit of the kidney V-H\(^+\)-ATPase is expressed in the intercalated cells of the kidney collecting ducts and it is also expressed in the cochlea and endolymphatic sac.6

Very little is known about the in vivo regulation of the various subunits of H\(^+\)-ATPase. Aldosterone is a potent regulator of distal H\(^+\) secretion.9–11 Angiotensin II (Ang II) is a potent regulator of luminal acidification and HCO\(_3\)^\(^-\) reabsorption along with Na\(^+\) and water in the proximal tubule.12 The presence of Ang II type I (AT\(_1\)) receptors in the distal tubule, including medullary collecting tubules, has been confirmed by both biochemical and molecular biological studies.13,14 In permeabilized cortical collecting duct segments, a specific dose-dependent inhibitory effect on the vacuolar H\(^+\)-ATPase in the presence of Ang II has been suggested.15 Weiner et al demonstrated that Ang II acts through a basolateral AT\(_1\) receptor to stimulate outer cortical collecting duct (charge-coupled device) luminal alkalinization via, at least in part, \(\beta\)-intercalated cell stimulation.16 By contrast, others have shown that Ang II stimulates H\(^+\)-
Plasma membranes from renal cortex or medulla were prepared as previously described.21,22 The kidneys were perfused with ice-cold phosphate-buffered saline (PBS) containing a cocktail of protease inhibitors via a retrograde cannula inserted in the abdominal aorta.23 After 30 to 50 mL had been perfused, the kidneys were removed and placed in fresh PBS on ice. The renal cortex and medulla were dissected free of each other and finely minced. The pieces were homogenized using a Duocyte tissue homogenizer in a buffer solution containing 300 mmol/L sucrose, 18 mmol/L Tris-HCl, 5 mmol/L EGTA, 4 μg/mL aprotinin, 5 μg/mL leupeptin, 2 μg/mL chymostatin, 2 μg/mL pepstatin, and 100 μg/mL AEBSF, with pH 7.4. The homogenates were centrifuged at 6000 g for 15 minutes at 5°C to yield a postmitochondrial fraction. The supernatants were then centrifuged at 45,000 g for 45 minutes at 5°C. The resulting crude membrane pellets were resuspended in 0.5 to 1 mL homogenizing buffer and stored at −80°C until used.

Western Blotting

Plasma membrane preparations were used for Western blotting.21 Protein concentrations were determined using the bicinchoninic acid protein assay (Biorad); 45 μg protein from renal cortical membranes or 20 μg protein from renal medullary membranes were mixed with 5× reducing sample buffer (Pierce) and then boiled for 5 minutes. Proteins were resolved by SDS polyacrylamide gel electrophoresis (SDS-PAGE, 7.5% gels). After electrophoresis, the proteins were transferred overnight onto nitrocellulose paper (Hybond+ Super; Amersham) by electroblotting.21,23

Protein detection was performed using double antibody staining. Before probing with the primary antibody, the nitrocellulose membrane was blocked for nonspecific protein binding by incubation for at least 1 hour with 5% powdered milk in PBS containing 0.1% Tween-20 (PBS-T). After blocking, the membrane was washed twice in 0.1% PBS-T and then probed overnight with rabbit polyclonal antibody against the B1 subunit of the H\textsuperscript{−}-ATPase diluted (1:1500) in 0.1% PBS-T plus 5% powdered milk. Antibody against the B1 and a4 subunits of the H\textsuperscript{−}-ATPase were diluted (1:1500) in 0.1% PBS-T plus 3% powdered milk. Antibody against the B1 and a4 subunits of the H\textsuperscript{−}-ATPase were a generous gift from Drs Karin Finberg and Fiona Karet. After probing, the membrane was subjected to multiple washes with PBS-T and then incubated for 1 to 2 hours with HRP-labeled donkey anti-rabbit IgG diluted (1:5000) in PBS-T plus 3% powdered milk. After incubation, the membranes underwent another series of washes with PBS-T. The bound antibody was then visualized using enhanced chemiluminescence detection (Amer- sham) and exposure to x-ray film. Densitometric quantification of the protein band corresponding to the B1 subunit of the H\textsuperscript{−}-ATPase, at ~56 kDa, was performed using an Eagle Eye II video capture system.21

Immunofluorescence

Kidneys from anesthetized rats were perfused free of blood with PBS and then immediately removed and placed in ice-cold saline solution. Half of one kidney was fixed for 6 hours by immersion in a solution containing: 4% paraformaldehyde, 10 mmol/L sodium periodate, 70 mmol/L lysine, and 5% sucrose. The fixative solution was prepared fresh daily. The fixed tissue was cyropreserved at −60°C until used for immunofluorescence staining. Before storage, the tissue was cryoprotected by immersion in a solution of 30% sucrose for at least 1 hour and then embedded with OCT on dry ice.

Before probing with the primary antibody, the nitrocellulose membrane was blocked for nonspecific protein binding by incubation for at least 1 hour with 5% powdered milk in PBS containing 0.1% Tween-20 (PBS-T). After blocking, the membrane was washed twice in PBS-T and then incubated with PBS-T plus 1% bovine serum albumin to block nonspecific background staining. Sections were then incubated with antibody against the B1 subunit of the H\textsuperscript{−}-ATPase (diluted 1:100) for 2 hours. Sections were then washed twice in PBS containing an additional 2.7% NaCl and then once with plain PBS, 5 minutes each. Sections were then incubated with fluorescein isothiocyanate-conjugated secondary antibody (goat anti-rabbit diluted 1:60) for 1 hour. Excess antibody was washed away and the sections were mounted with Vectashield antifading solution diluted with equal part 0.1 mol/L Tris base, pH 8.0, and then photographed.

Statistical Analysis

Data are presented as mean±SE. Differences between groups of paired rats were determined using a t test and Statview version 4.5 software. Comparisons of multiple groups were performed by ANOVA followed by Duncan multiple range test, using Super-ANOVA version 1.12 software. Differences between groups were considered statistically significant for P<0.05.

Results

Animal Characteristics

Sham surgical rats gained weight (11±2 grams) between the first surgical period and the time of euthanization 1 week later. ADX results in weight loss from salt and water wastage.18 Weight loss was attenuated in ADX rats by saline administration, but they still lost weight (~8±3 grams and ~5±3 grams in ADX and ADX+AngII, respectively).

Rats receiving Ang II for 1 week became hypertensive. Their blood pressure increased from 142±6 to 169±6 mm Hg (P<0.01) at the time of euthanization. Blood pressures in the other groups were not significantly different from each other at the end of the study (135±2 and 144±3 mm Hg in sham and ADX rats, respectively). Kidney weights and kidney to body weight ratios were not different between groups (data not shown).

Effect of ADX and Ang II on Renal B1 Protein Expression

Cell membrane preparations from renal medulla or cortex were used for Western blot analysis (Figure 1). Antibody against B1 protein recognized a band at ~56 kDa (Figure 1a...
and 1b). In the medulla of ADX rats, B1 expression was markedly reduced as compared with ADX rats infused with Ang II (Figure 1a). The relative amount of B1 protein in cell membranes isolated from the renal medulla of ADX rats was reduced as compared with sham rats (Figure 1a). In paired sets of rats, B1 protein abundance was >2-fold higher (2.19±0.47-fold; P<0.05) in renal medullas from rats with intact adrenal glands (shams, n=12) compared with those that had undergone bilateral ADX (n=12; Figure 2a).

In contrast to the renal medulla, the relative amount of B1 protein in cell membranes isolated from the renal cortex of ADX rats was not significantly altered as compared with sham surgical rats (Figure 3a). In paired sets of rats, B1 protein abundance was 1.15±0.15-fold (n=12; NS) in renal cortices from rats with intact adrenal glands compared with those that had undergone bilateral ADX (Figure 3a).

In separate groups of experiments, ADX rats were administered Ang II (28.8 µg/d per 100 grams of body weight) or vehicle (saline) for 7 days before euthanization. Antibody against B1 subunit recognized a band of 56 kDa. A, Representative Western blots of B1 protein from the kidney medulla of an ADX rat administered Ang II (lane 1), an ADX rat administered saline (lane 2), and a sham surgical rat (lane 3). B, Representative Western blot of B1 protein from the kidney cortex of an ADX rat administered Ang II (lane 4), ADX rats administered saline (lane 5), and a sham surgical rat (lane 6).

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The relative amount of α4 protein in cell membranes isolated from the renal medullas of ADX rats administered Ang II was not significantly altered as compared with ADX rats not administered Ang II (Figure 5b). In paired sets of rats, α4 protein abundance was $1.56 \pm 0.33$-fold ($n=8$, not significant) in renal medullas from ADX rats administered Ang II compared with those administered saline (Figure 5b). Similarly, Ang II did not significantly alter the relative amount α4 protein in cell membranes isolated from the renal cortex of ADX rats (Figure 6b). In paired sets of rats, α4 protein abundance was $1.59 \pm 0.34$-fold ($n=11$, not significant) in renal cortices from ADX rats administered a high dose of Ang II as compared with those given vehicle.

**Discussion**

Vacuolar-type (V-H\textsuperscript{+}-ATPases) are the major H\textsuperscript{+}-secreting proteins in the distal portion of the nephron and are involved in net H\textsuperscript{+} secretion (bicarbonate generation) or H\textsuperscript{+} reabsorption (net bicarbonate secretion).\textsuperscript{10,11} Screening for mutations in ATP6V1B1, the gene encoding the B1-subunit of H\textsuperscript{+}-ATPase, revealed 15 different mutations in kindreds in which almost all the affected individuals had documented distal tubular acidosis and bilateral sensorineural hearing loss.\textsuperscript{6} The majority of these mutations are likely to disrupt the structure or abrogate the production of the normal B1 subunit protein and result in a distinctive clinical phenotype characterized by metabolic acidosis caused by impaired distal H\textsuperscript{+} secretion. The B: isoform is part of the peripheral V\textsubscript{1} domain, with 2 isoforms the B2 isoform (ATP6V1B2) that is ubiquitously expressed,\textsuperscript{24} whereas in the kidney, the B1 subunit is amplified in intercalated cells of the late distal tubule, connecting segment and cortical and medullary collecting duct but is not expressed in the proximal tubule.\textsuperscript{25}

Studies on the hormonal regulation of vacuolar H\textsuperscript{+}-ATPase activity in the kidney have so far been limited to in vitro studies.\textsuperscript{17,26,27} Our study was designed to examine the in vivo expression of an H\textsuperscript{+}-ATPase subunit in a model of selective aldosterone deficiency (ADX rats maintained on glucocorticoid replacement) and to study the effect of exogenous Ang II on the in vivo expression of the B1 H\textsuperscript{+}-ATPase subunit. The model of Ang II excess that we used resulted in a significant elevation in blood pressure and used a dose that has been shown to result in increased levels of renal Ang II.\textsuperscript{19,23–30}

Our data showed an increase in the relative protein abundance of B1 subunit isoform of the H\textsuperscript{+}-ATPase after chronic

**Figure 4.** Immunofluorescence staining using antibodies against the 56-kDa H\textsuperscript{+}-ATPase B1 subunit in sections of rat kidney medulla. Magnification 400×. A, In collecting ducts from ADX rat administered Ang II, cells with intense apical immunoreactivity corresponding to α-intercalated cells were frequently seen. B, In collecting ducts from ADX rats, cells had a marked attenuation of staining. C, Cells from medullary collecting ducts from sham surgery rats showed intense staining on the apical membrane similar to that seen in tubules from Ang II infused rats, although staining does not appear to be as widespread within the tubules.

**Figure 5.** Densitometry of α4 protein obtained from rat medulla. Three groups of rats were treated as described in Methods. Densitometric values from paired sets of rats were normalized by setting the ADX saline value to 1. There were no differences between experimental groups and their paired controls. Data are expressed as mean±SE.

**Figure 6.** Densitometry of α4 protein obtained from rat cortex. Three groups of rats were treated as described in Methods. Densitometric values from paired sets of rats were normalized by setting the ADX saline value to 1. There were no differences between experimental groups and their paired controls. Data are expressed as mean±SE.
infusion of Ang II in the renal medulla of ADX rats. The expression of this H+ -ATPase is limited to intercalated cells in the collecting tubule. The antibody to the kidney isoform of the 56-kDa subunit stained intensely on apical membrane of intercalated cells from outer medullary collecting tubules and initial inner medullary collecting tubules in ADX infused with Ang II (Figure 4). By contrast, we did not find significant differences in B1 subunit of H+ -ATPase protein in cortical membrane preparations. The lack of difference in B1 protein expression renal cortex could simply reflect the predominance of renal proximal tubules, which do not express B1. However, B1 immunostaining in cortical collecting tubule segments was not increased by Ang II infusion to ADX rats as compared with saline ADX-paired rats not infused with Ang II. This suggests that hormonal regulation of B1 by aldosterone and Ang II occurs mainly at the level of medullary collecting ducts.

In contrast to the B1 subunit, the expression of the a4 subunit was not altered by either ADX or exogenous Ang II addition. Mutations in either the B1 or the a4 subunit have a distinctive inherited clinical phenotype characterized by the features of distal renal tubular acidosis.6,7 Interestingly, the a4 subunit is found in both the early segments of proximal tubules and in the intercalated cells in the kidney.8 The B1 subunit of the kidney H+ -V-ATPase, however, is expressed only in the intercalated cells of the kidney collecting tubules. Thus, one could speculate that regulation of the B1 subunit could be a mechanism by which hormones such as aldosterone and Ang II specifically regulate H+ -ATPase activity in the distal nephron.

The differential effect of Ang II and ADX on medullary and cortical collecting tubules deserves some comment. It has been previously demonstrated that medullary Ang II levels are higher than the cortical levels in normal rats and increase further in Ang II-infused hypertensive rats.28 An effect of elevated intrarenal Ang II to decrease AT1 receptor binding in the inner stripe of the outer medulla was also reported.29 A role for luminal Ang II in regulating reabsorptive function in distal nephron and collecting duct segments has been reported.29 In addition, the increase upstroke and trafficking of Ang II into renal endosomes mediated by AT1 receptors in renal cortex after chronic Ang II infusion29 could suggest that in renal cortex B1 regulation is under control of a mechanism(s) other than protein expression. Regulation of a4 and B1 subunits by trafficking but not protein expression has been demonstrated under conditions of acid base and electrolytes changes.6,12,17,27,31

It should be noted that different results regarding the effect of Ang II on H+ secretion have been reported at the functional level depending on the Ang II concentration and tubule segments studied.25-27 Microperfusion studies clearly showed a stimulatory effect of Ang II on bicarbonate reabsorption in distal tubules.36,37 Upregulation of H+ -ATPase activity by angiotensin II in intercalated cells via an AT1 receptor, which stimulated trafficking of the proton pump into the membrane has also been reported.30 In contrast, another study showed reduction in cortical collecting tubule vacuolar H+ -ATPase enzymatic activity in response to Ang II.15 Moreover, decreased H+ secretion was found in perfused medullary thick ascending limb and outer medullary collecting duct segments exposed to Ang II.38,39

Aldosterone increases H+ secretion, directly in the outer medullary collecting duct and indirectly via voltage effects secondary to increased sodium reabsorption in charge-coupled device segment.16,40 Our findings in ADX rats suggest that the removal of aldosterone results in a decrease in the relative abundance and expression of B1 H+ -ATPase in the medullary but not the cortical collecting tubule. These results suggest that aldosterone affects vacuolar H+ -ATPase activity in the collecting duct through different mechanisms that may be specific for segment and cell type. Our data show that some aldosterone actions may require protein synthesis of H+ -ATPase subunits. We recently showed that aldosterone increases a4 H+ -ATPase synthesis in a cell line of the collecting duct.39 A recent study showed nongenomic targeting of the a4 subunit of vacuolar H+ -ATPase to the apical membrane by aldosterone.27 An additional level of regulation via protein synthesis, however, may be important as well. Our results suggest that both aldosterone and Ang II provide such a mechanism of regulation in vivo at the level of the medullary collecting tubule. Interestingly, in this part of the nephron, the effects of aldosterone and Ang II are not sodium-dependent, whereas in the cortical collecting duct both aldosterone and Ang II, by contrast, affect H+ secretion by sodium-dependent mechanisms.40,41

We cannot rule out that the effects of Ang II were related to its hypertensive action, which may also affect hemodynamics of the medullary collecting duct. However, alterations in the medullary expression of the B1 subunit of the H+ -ATPase were seen in ADX rats administered Ang II, in which the blood pressure increased, but also in ADX rats administered vehicle, in which blood pressure was unchanged. Thus, the effects of Ang II and Ang II do not seem to be related to the hemodynamic effects of Ang II. Rather, it is more likely that both maneuvers exert direct effects on H+ -ATPase similar to those previously described on other acid-base and sodium transporters such as NHE335 and ENaC.42,43

Perspectives

In conclusion, our study shows that Ang II and ADX affect H+ -ATPase B1 subunit protein expression in medullary collecting ducts. Selective aldosterone deficiency created by adrenalectomy with glucocorticoid replacement resulted in downregulation in the expression of H+ -ATPase B1 subunit in medullary collecting ducts. In contrast, Ang II increases the expression of the B1 subunit of H+ -ATPase in the medullary collecting duct and thus may upregulate H+ secretion in this tubule segment. Such an action may be responsible, in part, for an increase in H+ secretion, independent of aldosterone, and thereby contribute to metabolic alkalosis in various hypertensive syndromes associated with Ang II overactivity.

References


5. Nelson N, Harvey WR. Vascular and plasma membrane proton-adenosine


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Hypertension. published online February 7, 2005;
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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