Acid loading in vivo and low pH in culture increase angiotensin receptor expression: enhanced ammoniagenic response to angiotensin II

Glenn T. Nagami, Jenny A. Chang, Megan E. Plato, and Rafael Santamaria

Nephrology Section 111L, Department of Medicine, Veterans Affairs Greater Los Angeles Healthcare System, and The David Geffen School of Medicine at the University of California Los Angeles, Los Angeles, California

Submitted 14 July 2008; accepted in final form 20 October 2008

Nagami GT, Chang JA, Plato ME, Santamaria R. Acid loading in vivo and low pH in culture increase angiotensin receptor expression: enhanced ammoniagenic response to angiotensin II. Am J Physiol Renal Physiol 295: F1864–F1870, 2008. First published October 22, 2008; doi:10.1152/ajprenal.90410.2008.—The proximal tubule defends the body against acid challenges by enhancing its production and secretion of ammonia. Our previous studies demonstrated an enhanced ammoniagenic response of the proximal tubule to ANG II added to the lumen in vitro after an in vivo acid challenge. The present study examined the effect of NH4Cl acid loading in vivo on renal cortical type 1 ANG II (AT1) receptor expression, the effect of low pH on AT1 receptor expression in a proximal tubule cell line, and their response to ANG II. A short-term (18 h) NH4Cl load in vivo resulted in increased renal cortical AT1 receptor mRNA expression and increased brush-border membrane AT1 receptor protein expression levels. Changing the cell culture pH from 7.4 to 7.0 for at least 2 h increased cell surface expression of AT1 receptors and enhanced the stimulatory effect of ANG II on ammonia production rates. This increased ammoniagenic response to ANG II and the early enhancement of cell surface expression induced by exposure of the cultured proximal tubule cells to pH 7.0 were prevented by treatment with colchicine. These results suggest that, after acid challenges, the enhanced ammoniagenic response of the proximal tubule to ANG II is, in part, mediated by increased AT1 receptor cell surface expression and that the enhancement of receptor expression plays an important role in the early response of the proximal tubule to acid challenges.

ammoniogenesis; angiotensin type receptor; cell surface; acidosis; colchicine

THE PROXIMAL TUBULE PLAYS an important role in defending the body against acid challenges by producing and secreting tNH3 (tNH3 = NH4+ + NH3) (21). Renal tNH3 production and excretion are enhanced in response to acid challenges (28, 29), and the major site of the increase in tNH3 production and secretion rates that occur in response to acid challenges is the proximal tubule (21).

ANG II plays an important role in the regulation of tNH3 production by the proximal tubule (4, 19, 20, 22). When tested in vitro, ANG II has concentration-dependent effects on tNH3 production and transport by the proximal tubule (19, 20). The stimulatory effect of ANG II on tNH3 production rates is mediated by type 1 ANG II (AT1) receptors, and this stimulatory effect is enhanced in proximal tubule segments derived from acid-loaded mice (22–24).

Using in vitro microperfused mouse proximal tubules, we previously demonstrated that a short-term (18 h) NH4Cl acid challenge in vivo resulted in an increased stimulatory effect of ANG II on tNH3 production (22). Nevertheless, it was unclear whether upregulation of the systemic renin-angiotensin system with acid loading (9, 10, 27) was responsible for the increased ammoniagenic response to ANG II of proximal tubule segments isolated from acid-loaded mice.

Because the ANG II concentrations within the proximal tubule lumen are very high compared with systemic concentrations (26, 31) and may exceed the binding constants for the ligand-ANG II receptor interaction, others have suggested that the response of the proximal tubule to ANG II in vivo may be determined, in part, by the level of expression of ANG II receptors (15). To examine further the effect of acid challenges on the effect of ANG II on tNH3 production rates, we studied the effect of 18 h NH4Cl loading on the protein and mRNA expression of AT1 receptors in mouse renal cortex and brush-border membranes (BBMs). To examine the effects of acid challenges on tNH3 production and AT1 receptor expression under more tightly controlled conditions, we used a proximal tubule cell line derived from dissected proximal tubule S1 segments from a large-T antigen transgenic mouse (14) to examine the effect of low pH on AT1 receptor expression. Our studies demonstrated that an 18-h NH4Cl load given to mice in vivo resulted in increased expression of AT1 receptor protein in renal cortical homogenates and in BBMs, and increased expression of renal cortical AT1a receptor mRNA. Our studies also demonstrated that exposing cultured proximal tubule cells to low (7.0) pH, serum-free medium increased total cellular and cell surface AT1 receptor protein expression and enhanced ammoniagenic response of the proximal tubule cells to ANG II. Both the enhanced ammoniagenic response to ANG II and the increased cell surface AT1 receptor protein expression levels observed when cells were exposed to low pH in culture were prevented by the disruption of the cellular microtubular network with colchicine.

METHODS

Animals. The studies performed were approved by the Animal Research Committee (Institutional Animal Care and Use Committee) at the Veterans Affairs Greater Los Angeles Healthcare System. Male Swiss-Webster mice (Hilltop, Scottsdale, PA) weighing 25–30 g were maintained on Purina Rodent Chow. Mice were provided 0.3 M NH4Cl in 2% sucrose or 2% sucrose alone (control) for a specified time. The mice were then anesthetized with isoflurane, and blood was obtained from the aorta for measurement of plasma bicarbonate concentration. Urine was obtained from the bladder for determination of tNH3 and creatinine as described previously (25), and urinary...
sodium concentrations were measured using an ion-selective electrode (Horiba).

Preparation of homogenates and BBM proteins. After mice were given 0.3 M NH₄Cl in 2% sucrose water or 2% sucrose water alone for 18 h, renal cortical tissue was dissected from two mouse kidneys, and homogenates were prepared by homogenization in mannitol buffer using a rotator-stator homogenizer, followed by slow-speed centrifugation to remove debris, as described previously (24). After an aliquot for total protein processing was taken, the remainder of the supernatant of the crude homogenate was used to prepare BBMs (24) as described by Booth and Kenny (1) and modified by Karniski et al. (12, 13). Briefly, the supernatant of the homogenate was treated with magnesium sulfate to bring the final concentration of magnesium to 11 mM. The preparation was mixed at 4°C for 20 min and then processed through a series of low-speed centrifugations (2,300 g for 8 min, 3,800 g for 4 min, 4,400 g for 8 min), each time saving the supernatant until a high-speed centrifugation step (14,000 g for 12 min) was performed to harvest the BBM vesicles. The pelleted vesicles were resuspended in RIPA lysis buffer [pH 7.4 phosphate-buffered saline with 1% Nε-tetrades F-40 (NP-40), 0.5% sodium deoxycholate, and 0.1% SDS] and kept on ice with intermittent mixing for 20 min before protein assay and storage. An aliquot of the BBM protein extract was digested with N-glycosidase (PNGase F; Promega). y-Glutamyltranspeptidase activity was enriched 8.5 ± 0.6-fold (n = 6) in the BBM preparation relative to the renal cortical homogenate.

Immunoblot analysis of AT1 receptors. Cell surface proteins bound to the agarose-streptavidin beads, BBM proteins, BBM proteins digested with PNGase F, renal cortical proteins, and total proximal tubule cell proteins were boiled for 5 min in reducing SDS gel loading buffer, separated by SDS-PAGE (NuPage, Invitrogen), and transferred to nitrocellulose membranes with a semidry electroblotter buffer, separated by SDS-PAGE (Invitrogen), and transferred to nitrocellulose membranes with a semidry electroblotter. The nitrocellulose membranes were blocked with a blocking buffer (0.1 M sodium malate, 0.15 M sodium chloride, pH 7.5, with 1% casein and 0.3% Tween 20), and the digoxigenin-labeled probes were detected using anti-digoxigenin alkaline phosphatase-conjugated Fab fragments in blocking buffer, followed by washes with plain malate buffer (0.1 M sodium malate, 0.15 M sodium chloride, pH 7.5) and exposure to CDP-Star chemiluminescence reagent. The chemiluminescence was captured on light-sensitive radiographic film (GE Healthcare) and was quantified with scanning densitometry.

S1 proximal tubule cells. S1 cells were originally isolated from the early portion of the proximal tubule from a large-T antigen transgenic mouse (14). The cells were grown to confluent monolayers in DMEM-Ham’s F-12 medium supplemented with 7% FBS (Hyclone) on 12-well tissue culture plates. The S1 cells were maintained in an incubator (Forma Scientific) in which the oxygen level was maintained at 35% and the carbon dioxide level at 5%. When tNH₃ production rates were studied, the cells were reincubated in serum-free medium for 24 h and then incubated for specified time periods (1, 2, 4 h) in serum-free medium with pH 7.0 or 7.4 (control). The reduction in pH was achieved by lowering the concentration of bicarbonate while maintaining a constant carbon dioxide concentration in the cell culture incubator. After incubation under the experimental conditions, the cells were washed twice with Krebs-Ringer bicarbonate (KRB) buffer, pH 7.4, containing 1 mM l-glutamine preincubated with 95% O₂-5% CO₂ at 37°C, then incubated in a known volume of KRB buffer with l-glutamine for 5 min in the cell culture incubator, and then washed and incubated again for another 5–10 min to measure the accumulation of tNH₃ in the assay buffer. Aliquots of assay buffer were treated with trichloroacetic acid to quench any further tNH₃ production and neutralized with potassium bicarbonate. In preliminary experiments, it was found that the rate of accumulation of tNH₃ in the assay buffer was linear over the first 15 min. In studies examining the acute effects of ANG II on tNH₃ production rates, ANG II was added at specified concentrations to the KRB assay buffer.

NH₃ assay. Total NH₃ in the neutralized urine samples was measured using a microfluorescence method that was based on the glutamic dehydrogenase reaction that converts 2-oxoglutarate, NH₃, and NADH to glutamate and NAD⁺. The reduction in NADH fluorescence was proportional to the tNH₃ present in the sample. Standards were processed in the same way as samples, and a standard curve was produced each time the assay was performed.

Cell surface expression of AT1 receptor in proximal tubule cells. Cell surface expression of the AT1 receptor was determined by labeling cell surface proteins with a cell membrane-impermeant labeling compound, sulfo-NHS-SS-biotin (Pierce). Cells exposed to pH 7.0 or 7.4 medium for specified times were washed with ice-cold PBS two times. The cells were then exposed for 2 h at 4°C to PBS containing sulfo-NHS-SS-biotin (0.5 mg/ml). After 2 h, the cells were washed with ice-cold Tris-buffered saline three times and extracted with pH 7.5 Tris (20 mM)-NaCl (150 mM) solution with 1% NP-40, 0.5% deoxycholate, and 0.1% SDS. The biotinylated proteins were collected using streptavidin-coated agarose beads (GE Healthcare) and thoroughly washed with PBS with 0.05% Tween 20 before electrophoresis. The total cellular proteins comprised the proteins in the cellular lysates without affinity purification on streptavidin beads. Measurements of diluted proteins were made by Bradford assay (Pierce).

Statistical analysis. Data are presented as means ± SE. Means of the various groups were compared using ANOVA with multiple comparisons [ANOVA Student-Newman-Keul’s method, GraphPad InStat software (GraphPad Software)].

RESULTS

Effect of 18 h NH₄Cl loading in vivo on AT1 receptor expression in mouse renal cortical tissue. Based on our previous studies that demonstrated an increased ammoniagenic
response to ANG II in proximal tubule segments isolated from mice exposed to an 18-h NH4Cl acid load, we examined the effect of 18 h NH4Cl loading on AT1 receptor expression in mouse renal cortical tissue. As we found before, at the end of 18 h acid loading with 0.3 M NH4Cl, serum bicarbonate concentration was not significantly different compared with non-acid-loaded controls (NH4Cl-treated: 23.0 ± 0.6 vs. control 23.3 ± 0.8 mM, n = 4) but markedly increased urine ammonia/creatinine excretion (NH4Cl-treated: 380 ± 30 μmol/mg creatinine vs. control: 100 ± 20, P < 0.01). To determine whether serum bicarbonate levels were lower in acid-loaded mice at an earlier time, we also examined the plasma bicarbonate concentration 8 h after initiation of the acid challenge and found that the plasma bicarbonate concentration was 20.7 ± 0.4 mM, which was significantly lower than the concentration observed in control mice (23.1 ± 0.5 mM) (n = 4, P < 0.05). These findings suggested that, after an 8-h acid load, mice were acidic compared with controls, but, after an 18-h acid load, the plasma bicarbonate concentration returned to normal, and this was associated with a high rate of tNH3 excretion.

Because the effective arterial volume could affect renal hemodynamics and AT1 receptor expression levels, we measured urinary sodium concentrations corrected for creatinine (μmol Na/mg creatinine). Urinary sodium/creatinine levels were not significantly different in controls compared with mice treated with NH4Cl for 18 h [123 ± 10 (control) vs. 115 ± 20 (NH4Cl-loaded), n = 3]. The absence of a significant sodium-retaining state after the acid challenge suggested the absence of a volume-depleted state.

Immunoblot analysis of AT1 receptor expression was performed on extracted cortical tissue homogenates and BBM proteins derived from acid-loaded and control mouse kidneys to examine the expression of AT1 receptor proteins. As shown in Fig. 1A, AT1 receptor protein was increased in both homogenates (total) and BBMs derived from acid-loaded mice. A difference in staining pattern was observed in the immunoblots of cortical tissue and BBM protein extracts, reflecting a difference in distribution in predominantly nonglycosylated AT1 receptor forms (double bands at ~42 kDa) in homogenates vs. the glycosylated form 64 kDa in BBMs (33). Thus the major immunodetected band of the BBM proteins migrated at 64 kDa, whereas the major band in the cortical tissue homogenates was 42 kDa. The relative AT1 receptor level of the major 42-kDa bands in the renal cortical homogenates from acid-loaded mice was 2.3 ± 0.3-fold (n = 4) higher than that of controls (P < 0.01), whereas the level of the major 64-kDa band in BBM preparations from acid-loaded mice was 1.9 ± 0.2 (n = 4) higher than that observed in controls (P < 0.05). Treatment of BBMs with peptide PNgase F resulted in the loss of the 64-kDa band and an increase in the abundance of the lower molecular forms, including the 42-kDa form. These studies demonstrated that total cellular and apical BBM protein expression levels of AT1 receptors were enhanced by an 18-h NH4Cl acid load and that the 64-kDa protein band that was upregulated in BBMs with acid loading represented a glycosylated form of the receptor.

To determine whether differences in mRNA expression levels could contribute to the differences in protein expression observed in renal cortical tissue from acid-loaded and non-acid-loaded mice, we performed RNase protection assays using digoxigenin-labeled AT1a receptor and β-actin-specific cRNA probes. The AT1a receptor mRNA expression level was higher in cortical tissue from mice receiving the NH4Cl-acid load compared with levels observed in tissue from control mice (Fig. 1B depicts a representative RNase protection assay). When β-actin mRNA expression levels were used as a reference, with acid loading, the relative AT1a receptor mRNA expression level in renal cortical tissue was 2.3 ± 0.2-fold (n = 3) higher than in corresponding controls (P < 0.05).

Effect of low pH in culture on AT1 receptor expression in proximal tubule cells. We used a cell line of proximal tubule cells derived from a dissected S1 proximal tubule from a large-T antigen transgenic mouse (14) to determine the effect of low pH in cultured cells on AT1 receptor expression and on the effect of ANG II on tNH3 production rates. S1 cells were incubated in serum-free pH 7.4 or 7.0 medium for 1, 2, or 4 h, and cell surface expression of AT1 receptor was examined by immunoblot analysis of surface biotinylated proteins (Fig. 2A depicts a representative immunoblot). Incubation at low pH resulted in increased AT1 receptor cell surface protein expression levels at 2 h (2.2 ± 0.2-fold increase vs. pH 7.4, n = 4, P < 0.01) and at 4 h (2.4 ± 0.2-fold vs. pH 7.4, n = 4, P < 0.01), but not after a shorter incubation period of 1 h. On the other hand, low pH did not increase total cellular AT1 receptor protein expression (detected in the 42-kDa bands) until 4 h (1.8 ± 0.2-fold increase vs. pH 7.4, n = 4, P < 0.05) of incubation (Fig. 2B depicts a representative blot). These studies demonstrated that cultured S1 cells express AT1 receptors on their cell surface and that low pH enhanced cell surface expression of the AT1 receptors before it enhanced total cellular AT1 receptor abundance.

Effect of exogenous ANG II on tNH3 production by S1 cells. Because proximal tubule segments increase their tNH3 production rates in response to ANG II and proximal tubule segments derived from acid-loaded mice display a heightened ammoniagenic response to acute exposure to ANG II in vitro, we determined whether ANG II increased tNH3 production in cultured S1 cell monolayers (n = 6 for each group) and whether preexposure of S1 cell monolayers to low pH resulted...
AT1 receptor protein expression levels at the 42-kDa bands were increased by II had no effect on tNH3 production (5.3 7.0 for only 1 h did not result in an enhanced stimulation of the 6.7 0.2-fold increase vs. pH 7.4, n = 4, P < 0.01). B: total cellular AT1 receptor protein expression levels at the 42-kDa bands were increased by low pH at 4 h (1.8 0.2-fold increase vs. pH 7.4, n = 4, P < 0.05), but not at 2 h.

in an enhanced ammoniagenic response to ANG II (n = 6 for each group). We preincubated S1 cell monolayers at pH 7.4 or 7.0 for 2 h in serum-free medium and subsequently measured their rates of tNH3 production in KRB buffer containing 1 mM L-glutamine with different concentrations of ANG II. Acute exposure of S1 cell monolayers to ANG II increased tNH3 production rates in cells incubated at pH 7.4 in a concentration-dependent fashion (Fig. 3). Acute exposure to 10^{-11} M ANG II had no effect on tNH3 production (5.3 0.2, n = 6), whereas incubation with 10^{-10} or 10^{-9} M ANG II significantly stimulated tNH3 production rates (7.1 0.2 and 7.0 0.3, respectively (P < 0.01 vs. no ANG II, n = 6)). Incubation of S1 cells with 10^{-7} M ANG II had no effect on tNH3 production rates compared with the 0 ANG II control (5.9 0.2, n = 6). As shown in Fig. 4, incubating the cells at pH 7.0 for 2 h increased the stimulatory effect of ANG II on tNH3 production rates such that addition of 10^{-9} M ANG II had a significantly greater effect on cells incubated at pH 7.0 for 2 h compared with cells incubated at pH 7.4 [8.9 0.2 nmol·min^{-1}·mg protein^{-1}] for pH 7.0 with ANG II vs. 7.1 0.2 for pH 7.4 with ANG II (P < 0.01, n = 6)]. Basal rates of ammonia production were not affected by 2 h preexposure of S1 cells to pH 7.4 vs. 7.0 if not stimulated by ANG II (5.7 0.2, pH 7.4, without ANG II and 5.4 0.3, pH 7.0, without ANG II). Incubation of cells at pH 7.0 for only 1 h did not result in an enhanced stimulation of the tNH3 production rate by ANG II (6.9 0.3 at pH 7.0 for 1 h vs. 7.2 0.2 at pH 7.4 for 1 h, P > 0.4). Exposing cells to losartan (10^{-5} M) concurrently with ANG II significantly inhibited the stimulatory effect of ANG II (10^{-9} M) on tNH3 production rates (ANG II + Losartan) at pH 7.0 (6.1 0.3 nmol·min^{-1}·mg protein^{-1}, P < 0.05). Losartan in the absence of ANG II had no significant effect on tNH3 production rates (data not shown).

**Effect of colchicine on tNH3 production rates in response to ANG II** and on AT1 receptor cell surface expression. The role of the microtubules on the ammoniagenic response of S1 cells to ANG II (10^{-9} M) after preincubation at pH 7.4 or 7.0 was examined. S1 cell monolayers (n = 6 for each group) were preincubated at pH 7.4 or 7.0 medium for 2 h in the presence or absence of colchicine (10^{-5} M) and then exposed to 10^{-9} M ANG II to measure its acute effects on tNH3 production rates (Fig. 5). The tNH3 production rates were higher in S1 cells exposed to ANG II after preincubation at pH 7.0 (8.8 0.2 nmol·min^{-1}·mg protein^{-1}) compared with cells preincubated at pH 7.0 with colchicine (7.4 0.2, P < 0.05, n = 6 for each group).
group), cells preincubated at pH 7.4 (7.1 ± 0.3, P < 0.01), and to cells preincubated at pH 7.4 with colchicine (7.3 ± 0.2, P < 0.05). Thus exposure of proximal tubule cells to colchicine during the low (7.0) pH preincubation period prevented the heightened ANG II-stimulated rate of tNH₃₃ production observed with low pH preincubation in the absence of colchicine. On the other hand, colchicine had no discernable effect on ANG II-stimulated ammoniagenesis in cells preincubated at pH 7.4.

Exposure of cells to 10⁻⁵ M colchicine at pH 7.0 also blunted the increase in cell surface AT₁ receptor expression observed at pH 7.0 such that cell surface expression was reduced back to levels observed at pH 7.4 with colchicine (Fig. 6). S₁ cells exposed to pH 7.0 without colchicine for 2 h displayed a 1.8 ± 0.1-fold increase in AT₁ receptor cell surface expression compared with cells incubated in pH 7.0 with colchicine (P < 0.05, n = 4 for each group). Cell surface AT₁ receptor expression was also 1.9 ± 0.2-fold higher (P < 0.05) in cells exposed to low pH without colchicine for 2 h compared with cells incubated at pH 7.4 with colchicine and 2.5 ± 0.2-fold higher (P < 0.01) compared with cells incubated in pH 7.4 medium without colchicine. Colchicine added to the pH 7.4 medium resulted in a small but consistent increase cell surface receptor expression (1.4 ± 0.1-fold vs. pH 7.4 alone, P < 0.05).

**DISCUSSION**

ANG II is an important regulator of acid-base transport and metabolism in the proximal tubule (4, 6–8, 18, 19). We have previously demonstrated that ANG II enhances the ability of the proximal tubule to respond to acid loads by increasing tNH₃₃ production and secretion (22). Furthermore, acid loads also increase the stimulatory effects of ANG II on tNH₃₃ production and secretion by the proximal tubule such that proximal tubules derived from mice exposed to an 18-h acid load display increased rates of tNH₃₃ production and secretion with addition of ANG II to the luminal fluid compared with tubules from non-acid-loaded controls (22).

ANG II is present in the proximal tubule lumen in vivo in substantial concentrations (2, 26, 31) that greatly exceed blood levels, and it has been proposed that, because the concentration appears to exceed the binding coefficient of the AT₁ receptor for ANG II, variations in the level of receptor expression can modulate AT₁ receptor-mediated responses to ANG II (15). To explore the mechanisms for the enhanced ammoniagenic response to ANG II observed with acid challenges, we measured AT₁ receptor expression levels in renal cortical tissue and BBMs derived from mice exposed to acid loads and in cultured S₁ proximal tubule cells exposed to reduced pH of the medium.

We found that AT₁ receptor protein expression was increased in BBMs. The increase in immunostaining bands observed in cortical tissues and BBMs was different in that the major band of increased expression was the 64-kDa band in BBM samples, whereas the lower nonglycosylated 42-kDa bands were increased in the cortical homogenates. Work by others has demonstrated that AT₁ receptors are glycosylated with a major glycosylated band migrating at ~64 kDa (11, 33) and that glycosylation is important for the efficient movement of the receptor to the cell surface (5, 11, 16, 17). Thus the predominance of a glycosylated form of the AT₁ receptor associated with the BBM is not surprising. Our previous observations demonstrated an enhanced response to ANG II presented to the luminal aspect of microperfused proximal tubule segments from acid-loaded mice (22), and the enhanced AT₁ receptor expression in BBMs would correlate with this increased luminal response. An enhanced expression level of AT₁ receptor mRNA was also observed and could account, at least in part, for increased AT₁ receptor expression in renal tissue derived from acid-loaded mice.

Although the plasma bicarbonate levels, after 18 h, were no different in acid-loaded mice compared with non-acid-loaded controls, urinary tNH₃₃ excretion and renal AT₁ receptor expression were increased at that time. This might have indicated a pH-independent effect of acid loading on AT₁ receptor expression. However, when plasma bicarbonate concentrations were measured after a shorter duration of exposure to NH₄Cl (8 h), we found that they were significantly lower in the acid-loaded mice compared with controls. In other words, during the course of acid loading, mice initially developed metabolic acidosis that, at least temporarily, resolved at 18 h as the result of increased urinary tNH₃₃ excretion. Therefore, the initial presence of metabolic acidosis could have played a role in the enhancement of urinary tNH₃₃ excretion and upregulation of AT₁ receptors.

Another factor that could affect AT₁ receptor expression in the kidney is volume depletion. We have observed that mice given drinking water containing 2% sucrose consume approximately equal amounts of fluid whether or not NH₄Cl is present in the solution. In the present study, the urinary sodium-to-creatinine ratio showed that sodium excretion levels after 18 h treatment with NH₄Cl-sucrose water and with sucrose water alone were not significantly different. The absence of signifi-
cant sodium retention suggested the absence of a major degree of volume depletion in the acid-loaded mice at 18 h.

To distinguish between systemic hemodynamic and hormonal effects and the effects of pH per se on AT$_1$ receptor expression, we turned to a cell culture model. The present studies demonstrated that an S$_1$ cell line derived from the proximal tubule of a large-T antigen transgenic mouse expressed AT$_1$ receptors on their cell surfaces and that the levels of cell surface receptor expression were enhanced after 2 h, but not after 1 h, of exposure to a reduced pH in culture. The enhancement of cell surface receptor expression was associated with an enhanced ammoniagenic response of these cells to ANG II. Increased total AT$_1$ receptor protein expression was not observed until after 4 h of exposure to low pH, suggesting a role for enhanced net membrane insertion of premade receptor in the cell membrane in the early response to low pH as well as a later induction of increased amounts of AT$_1$ receptor protein. All of the observed changes in AT$_1$ receptor protein expression occurred in serum-free medium, suggesting that the low pH-induced changes in protein and mRNA expression occurred independently from the influence of exogenous hormones. Because the enhancement of AT$_1$ receptor protein and mRNA expression occurred in the cell culture model, the potential effects of systemic hormonal and hemodynamic changes on receptor expression were excluded, thereby supporting an effect of pH per se on AT$_1$ receptor expression.

Movement of AT$_1$ receptors in and out of the cell surface is well-described and involves interactions with microtubules and other cytoskeleton elements (15, 29, 32). In the present study, the addition of the microtubular depolymerizing agent colchicine prevented the enhanced response of proximal tubule cells preincubated at pH 7.0 to ANG II and also disrupted the enhancement of cell surface expression of AT$_1$ receptors by low pH. The reduction of cell surface expression at low pH by colchicine could have resulted from a reduced rate of insertion or an enhanced rate of removal. It has been suggested by Kolb et al. (15) that the microtubular network allows externalization of the AT$_1$ receptor at the luminal membrane of the proximal tubule and that the rate of insertion may serve as the limiting factor for ANG II effects at this site, since ANG II concentrations in the lumen are above the kilodaltons for its receptor (3). Our results showing a small effect of colchicine on cell surface expression at normal (7.4) pH also suggested that colchicine may have had an inhibitory effect on receptor internalization under these conditions.

The upregulation of AT$_1$ receptor expression by acid loading in vivo or low pH in culture may be associated with an enhanced action of ANG II that is present at very high concentrations in the kidney (2, 15, 31). In previous studies, we demonstrated that the stimulation of ammoniagenesis by ANG II was mediated, at least in part, by a rise in intracellular calcium concentration (20). Blocking the ANG II-induced rise in intracellular calcium prevented the stimulation of tNH$_3$ production. It is possible that preexposure of proximal tubule cells to low pH in culture or acid challenge in vivo may augment the effects of ANG II on intracellular calcium signaling via upregulation of AT$_1$ receptors. This possibility may be examined in future studies.

In summary, our studies demonstrated that 18 h acid loading in vivo enhances AT$_1$ receptor protein and mRNA in renal cortical tissue and receptor protein expression in BBMs. These results could explain our previous finding that in vivo acid loads are associated with increased response of proximal tubule segments to ANG II. Acute exposure of cultured S$_1$ proximal tubule cells to ANG II increased tNH$_3$ production rates, and preexposure of the cells to low pH in culture significantly enhanced the stimulatory effect of ANG II on ammoniagenesis. This enhanced response of S$_1$ cells to ANG II corresponded to a low pH-induced enhancement of AT$_1$ receptor cell surface expression involving the microtubular network. Although total cellular AT$_1$ receptor expression was eventually enhanced with more prolonged exposure to low pH in culture, the early enhancement of AT$_1$ receptor cell surface expression and enhanced response to ANG II occurred before a detectable increase in total cellular AT$_1$ receptor expression and suggested that redistribution of AT$_1$ receptors to the cell surface could represent an early response to low pH. The major physiological implication of the results of this study is that the stimulatory effects of ANG II on acid and ammonia secretion by the proximal tubule are further enhanced through the upregulation of AT$_1$ receptors in the proximal tubule by acid challenges.

ACKNOWLEDGMENTS

We thank Evelyn M. Warech and Raymond Beyda for technical assistance. Current address for R. Santamaria: Servicio de Nefrología, Hospital Universitario Reina Sofia, Córdoba, Spain.

GRANTS

This work was supported by research funds (Merit Review Award) from the Department of Veterans Affairs. R. Santamaria was supported by the Instituto de Salud Carlos III, Spain.

REFERENCES


