Influence of ANG II on cytoplasmic sodium in cultured rabbit nonpigmented ciliary epithelium

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Hou, Yining, and Nicholas A. Delamere. Influence of ANG II on cytoplasmic sodium in cultured rabbit nonpigmented ciliary epithelium. Am J Physiol Cell Physiol 283:C552–C559, 2002.—Angiotensin (ANG) II receptors have been shown to be localized in the ciliary epithelium bilayer, the tissue responsible for aqueous humor secretion. In the rabbit eye, ANG II appears to inhibit H^+\text{-ATPase}-mediated proton export. Thus Ang II (10 nM) did not alter the baseline cytoplasmic pH (pHi) but reduced pHi in cells that were also exposed to 10 μM DMA. Consistent with the notion of H^+\text{-ATPase} inhibition in ANG II-treated NPE, bafilomycin A1 (100 nM) (BAP) and ANG II were both observed to suppress the pHi increase that occurs upon exposure to a mixture of epinephrine (1 μM) and acetylcholine (10 μM DMA). In summary, these results suggest that ANG II causes H^+\text{-ATPase} inhibition and an increase in cell sodium due to activation of Na^+\text{/H}^+ exchange.

ANGIOTENSIN-CONVERTING ENZYME inhibitors and angiotensin (ANG) II receptor antagonists are able to cause a reduction of intraocular pressure (1, 8, 9, 23), and the exposure of cultured human NPE to ANG II was observed to increase cytoplasmic calcium concentration (14). In situ hybridization showed mRNA for both AT1 and AT2 receptor subtypes in rat ciliary body (35).

The ability of ANG II to alter sodium transport has been extensively documented. In several tissues, Na^+\text{-K}^+\text{-ATPase} is inhibited following AT receptor activation (2, 20, 24). In the present study, we examined the influence of ANG II on cytoplasmic sodium in cultured rabbit NPE. An increase in the rate of Na^+\text{-K}^+\text{-ATPase}-mediated ^86\text{Rb} transport observed in ANG II-treated NPE cells appeared to be the result of a rise of cytoplasmic sodium concentration. In the kidney, ANG II is also reported to change the activity of H^+\text{-ATPase}-mediated proton export (15, 31, 32).

MATERIALS AND METHODS

Chemicals. 2′,7′-Bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF)-AM, fura 2-AM, sodium-binding benzofuran isophthalate (SBFI)-AM, and Pluronic F-127 were obtained from Molecular Probes (Eugene, OR). All other chemicals were purchased from Sigma (St. Louis, MO). Materials insoluble in water were dissolved in minimum volume of dimethyl sulfoxide (DMSO), 20% Pluronic F-127 in DMSO, or ethanol (<0.1% final concentration). An equal amount of DMSO or ethanol was added to control solutions.

Cell culture. The NPE cell line used in this study was a kind gift from Dr. M. Coca-Prados (Yale University, New Haven, CT). The cells were derived from SV40 virus-transformed rabbit nonpigmented ciliary epithelium and have been used previously in studies of pHi regulation and active ATP hydrolysis measurements. H^+\text{-ATPase} activity (bafilomycin A1-sensitive ATP hydrolysis) was reduced significantly in cells that had been pretreated 10 min with 10 nM ANG II. In summary, these studies suggest that ANG II causes H^+\text{-ATPase} inhibition and an increase in cell sodium due to activation of Na^+\text{/H}^+ exchange.

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Measurement of cell sodium by atomic absorption spectrophotometry. Cell monolayers were washed with ice-cold isotonic magnesium chloride solution (100 mM MgCl₂, adjusted to pH 7.4 with Tris base). The magnesium chloride solution was then removed, and the cells were lysed by adding 200 μl of 30% nitric acid to each well. After this, 1.8 ml of deionized water was added to each well, and the sodium content of the diluted cell lysates was measured by using an atomic absorption spectrophotometer (Perkin-Elmer, Norwalk, CT) at a wavelength of 566.5 nm.

Measurement of pH, by digital fluorescence microscopy. The fluorescent pH-sensitive dye BCECF was used to measure pH in cells that were continuously superfused at a rate of 1 ml/min with Krebs solution with the following composition: 119 mM NaCl, 4.7 mM KCl, 1.2 mM KH₂PO₄, 25 mM NaHCO₃, 2.5 mM CaCl₂, 1 mM MgCl₂, and 5.5 mM glucose at pH 7.4, equilibrated with 5% CO₂–95% air. Potassium-rich Krebs solution was prepared by increasing the concentration of KCl to 80 mM and decreasing the concentration of NaCl by 80 mM. To load the cells with fluorescent dye, the cells were incubated for 1 h in Krebs solution containing 10 μM BCECF-AM under a humidified 5% CO₂–95% air at 37°C. After being loaded, the cells were washed three times with Krebs solution, and the petri dish was mounted on the stage of a fluorescence microscope (Zeiss, Thornwood, NY) equipped with a digital imaging system (Attofluor Instruments, Rockville, MD). The microscope stage was warmed to 37°C by a water jacket, and a flow-through temperature controller (Warner Instrument, Hamden, CT) was used to set the temperature of the incoming superfusate to 37°C. The fluorescence intensity of BCECF was measured by using an emission wavelength of 520 nm and alternating dual-excitation wavelengths of 460 and 488 nm. The relationship between pH, and the ratio of fluorescence intensity at 460 nm to that at 488 nm was calibrated at the end of each experiment. Cells were first exposed to a potassium-rich buffer containing 10 μM nigericin, which mediates K/H exchange and thereby equilibrates the extracellular pH and intracellular pH. The potassium-rich buffer contained 110 mM KCl, 20 mM NaCl, and 100 mM NaCl, and a 20 mM buffer selected to control pH. 2-(N-morpholino)ethanesulfonic acid (MES; pH₅₆ = 6.1) was used to set pH in the range 6.0–6.5; piperazine-N,N'-bis(2-ethanesulfonic acid) (PIPS; pH₅₆ = 6.8) was used to set pH at 7.0; N₂-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPS; pH₅₆ = 7.5) was used to set pH at 7.4; and N-tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid (TAPS; pH₅₆ = 8.4) was used to set pH at 8.4.

Measurement of cytoplasmic sodium concentration by digital fluorescence microscopy. The fluorescent dye SBFI was used to measure cytoplasmic sodium concentration. SBFI-AM was dissolved in 20% Pluronic F-127 in DMSO and added to the Dulbecco’s modified Eagle’s cell culture medium for 3 h at a final concentration of 10 μM SBFI-AM, <0.1% Pluronic F-127, and <0.5% DMSO. The methodology was similar to that described for pH measurements. The alternating excitation wavelengths were 340 and 380 nm. To calibrate the signal at the end of each experiment, the cells were permeabilized by exposure to Krebs solution containing 10 μM nigericin, 5 μM monensin, and 5 μM gramicidin and a range of sodium concentrations. The fluorescence ratio signal was thus measured in the permeabilized cells equilibrated to different external sodium concentrations.

Measurement of cytoplasmic calcium by digital fluorescence microscopy. Cytoplasmic calcium concentration was measured using the calcium-sensitive dye fura 2 with alternating excitation wavelengths of 344 and 380 nm. The methodology was similar to that described for pH measurements.

The relationship between cytoplasmic calcium and the ratio of fluorescence intensity at 344 nm to that at 380 nm was calibrated at the end of each experiment by first adding 1 μM ionomycin to the superfusate to permit calcium equilibration with the external solution to obtain the maximum fluorescence ratio. EGTA (30 mM) was then added to the superfusate in the continued presence of ionomycin to obtain minimum fluorescence ratio.

Measurement of H⁺–ATPase activity. H⁺–ATPase activity was determined by using a modification of the technique described by Tojo and coworkers (30) in which ATP hydrolysis is coupled to the oxidation of NADH. Before the ATP hydrolysis stage of the experiment, cell monolayers were incubated in Krebs solution, and some cells were also exposed to 10 nM ANG II for 10 min. After this, the Krebs solution was replaced by 100 μl of assay buffer containing 100 mM NaCl, 66.7 mM NH₄Cl, 3.7 mM MgCl₂, 2 mM CaCl₂, 50 mM imidazole, 5 mM glucose, and 0.05% bovine serum albumin. The samples were immediately frozen for 30 min and then thawed to permeabilize the cells. Each sample then received 100 μl of reaction solution containing 100 mM NaCl, 66.7 mM NH₄Cl, 50 mM imidazole, 3.7 mM MgCl₂, 7.5 mM sodium octyl glucoside, 3.3 mM sodium fluoride, 2 mM EGTA, 12 mM ouabain, 0.6 mM phosphonopyruvate, and 9.6 U/ml pyruvate kinase. Half the samples also received bafilomycin A₁ added to a final concentration of 100 nM. Bafilomycin A₁ is a specific inhibitor of H⁺–ATPase activity (4). The samples were incubated at 37°C in a shaking water bath for 40 min. At the end of the ATP hydrolysis period, 1 ml of a solution containing 2.5 mM NADH and 6 U/ml lactate dehydrogenase in 0.1 N potassium phosphate buffer was added for 40 min, and then 150 μl of 30% ice-cold trichloroacetic acid was added. The supernatant was then removed, and the amount of NADH oxidized by pyruvate was measured by using a fluorometer to quantify the decrease in emission at 460 nm with an excitation wavelength of 340 nm. The cells remaining in each well were digested in 0.5 N NaOH, and an aliquot was used to measure protein by using a colorimetric Bio-Rad assay (Bio-Rad, Hercules, CA). Because there is a 1:1 stoichiometric relationship between the amount of NADH oxidized and the amount of ADP generated as the result of ATP hydrolysis, H⁺–ATPase activity was calculated from the difference in the amount of NADH oxidized in the presence or absence of the H⁺–ATPase inhibitor bafilomycin A₁ (100 nM). H⁺–ATPase activity is expressed as picomoles of ATP hydrolyzed per milligram of protein per minute.

Data analysis. Student’s t-test was used for the statistical analysis. Values of P < 0.05 were considered significant.

RESULTS

ANG II increases the cytoplasmic sodium concentration. Cultured nonpigmented ciliary epithelial cells were preloaded with SBFI and then exposed to ANG II at a concentration of 1 nM. A progressive increase of cytoplasmic sodium concentration was observed shortly after ANG II addition (Fig. 1A). In separate experiments, the sodium content of NPE cell monolayers was measured by using atomic absorption spectrophotometry. Cells were exposed for 30 min to ANG II at concentrations ranging from 0.1 pM to 10 nM. A significant increase in sodium content occurred in cells exposed to 1 nM ANG II (Fig. 1B).
Dimethylamiloride. In other tissues it has been suggested that Na\(^+\)/H\(^+\) exchanger activity may be altered by ANG II. To examine the possible contribution of the Na\(^+\)/H\(^+\) exchanger to the ANG II-induced increase of cell sodium content, cells were exposed to ANG II in the presence of 10 \(\mu\)M dimethylamiloride (DMA), an inhibitor of Na\(^+\)/H\(^+\) exchange (6) that is also known to inhibit epithelial sodium channels when used at higher concentrations (5, 33). In the presence of DMA, ANG II failed to elicit a significant increase of cell sodium content (Fig. 1B).

Involvement of ANG II receptors. To test whether the mechanism involves activation of ANG II receptors, some cells were exposed to ANG II in the presence or absence of saralasin, an antagonist for both AT\(_1\) and AT\(_2\) receptors. Saralasin suppressed the sodium increase in ANG II-treated cells in a dose-dependent manner (Fig. 2A).

Fig. 1. A: a typical experiment showing the influence of angiotensin (ANG) II on cytoplasmic sodium concentration. Cytoplasmic sodium concentration was measured by fluorescence in cells loaded with the sodium-sensitive dye sodium-binding benzofuran isophthalate. After a stable cytoplasmic sodium concentration baseline was established, cells were exposed to ANG II (1 nM). B: influence of dimethylamiloride (DMA) on the cytoplasmic sodium response to ANG II. Cytoplasmic sodium content was measured using atomic absorption spectrophotometry. Cells were exposed to ANG II (0.1 pM-10 nM) in the presence (●) or absence (▲) of DMA 10 \(\mu\)M. Data are means ± SE \((n = 12)\). *Significant difference \((P < 0.01)\) from the sodium content measured in the presence of 0.1 pM ANG II.

Fig. 2. A: influence of saralasin on cytoplasmic sodium in cells exposed to 10 nM ANG II. Cytoplasmic sodium content was measured using atomic absorption spectrophotometry. Cells were exposed to saralasin (1 pM-10 \(\mu\)M) in the presence of 10 nM ANG II. Data are means ± SE \((n = 12)\). Based on a curve fit to the data (solid line), the IC\(_{50}\) for saralasin was 3.72 ± 0.73 nM. B: influence of losartan on cytoplasmic sodium in cells exposed to 10 nM ANG II. Cells were exposed to losartan (1 pM-1 \(\mu\)M) in the presence of 10 nM ANG II. Data are means ± SE \((n = 6)\). Based on a curve fit to the data (solid line), the IC\(_{50}\) for losartan was 0.57 ± 0.15 nM. C: the influence of ANG II on cytoplasmic sodium in the presence or absence of 100 \(\mu\)M PD-123319 (PD). Cells were exposed to control Krebs solution (no ANG II or PD), PD (100 \(\mu\)M) alone, or ANG II (10 nM) in the presence of 100 \(\mu\)M PD. Data are means ± SE \((n = 6)\). *Significant difference from the control sodium content \((P < 0.01)\).
Based on a curve fit to the data shown in Fig. 2A, the IC₅₀ for saralasin was 3.7 ± 0.7 nM. Losartan, an AT₁-selective receptor antagonist, also inhibited the cytoplasmic sodium increase in ANG II-treated cells (Fig. 2B). The calculated IC₅₀ for losartan was 0.57 ± 0.15 nM. In contrast, PD-123319 (100 μM), an AT₂ receptor antagonist, failed to prevent the rise of sodium that followed ANG II exposure (Fig. 2C).

Na⁺-K⁺-ATPase-mediated sodium-potassium transport. The ability of ANG II to cause an increase in cell sodium concentration suggests that Na⁺-K⁺-ATPase-mediated ion transport might be stimulated in cells exposed to ANG II. To test this, ouabain-sensitive potassium uptake was measured as ⁸⁶Rb uptake in the cells treated with ANG II. In the presence of 10 nM ANG II, the rate of ⁸⁶Rb uptake was stimulated by ~40% (Fig. 3). In sharp contrast, ANG II failed to increase the rate of ⁸⁶Rb uptake in cells that were simultaneously exposed to 10 μM DMA (Fig. 3).

pHᵢ. Studies were conducted to examine the influence of ANG II on pHᵢ. Cells were preloaded with the pH-sensitive dye BCECF before being exposed to 10 nM ANG II. The resting pH was 7.1 ± 0.14 (mean ± SE; n = 6 experiments). The addition of ANG II failed to cause a detectable pH change (Fig. 4A). However, in the presence of DMA, ANG II produced cytoplasmic acidification. Added alone, 10 μM DMA reduced pHᵢ from 7.09 ± 0.05 to a new stable value of 6.77 ± 0.07 (Fig. 4B). Exposure of the cells to 10 nM ANG II in the continued presence of DMA caused a further pH reduction to 6.45 ± 0.04.

Bafilomycin A₁. In a previous study, bafilomycin A₁ was found to reduce pHᵢ in DMA-treated cells (12). The similarity between the pH responses of DMA-treated cells to ANG II and bafilomycin A₁ suggests that H⁺-ATPase might be inhibited when cells are exposed to ANG II. Consistent with this notion, bafilomycin A₁ and ANG II were both found to inhibit the pHᵢ increase that occurs when NPE cells are exposed to a mixture of epinephrine and acetylcholine (Fig. 5, A and B). Similarly, ANG II and bafilomycin A₁ both significantly (P < 0.01) reduced the magnitude of the pHᵢ increase that occurs on cell depolarization by potassium-rich solution; in control cells, exposure to 80 mM KCl for 5 min caused a pHᵢ increase of 0.58 ± 0.12 pH unit, whereas in the presence of ANG II (10 nM) or bafilomycin A₁ (100 nM) the pHᵢ increase was 0.18 ± 0.11 or 0.15 ± 0.09, respectively (means ± SE; n = 6). Like ANG II, bafilomycin A₁ did not directly alter pHᵢ (Fig. 5C). Moreover, pHᵢ was also found to remain stable when ANG II was added in the continued presence of bafilomycin A₁ (Fig. 5C).

If exposure of cultured NPE to ANG II causes inhibition of H⁺-ATPase, then it is predicted that ANG II and bafilomycin A₁ may have similar effects on cell sodium content. This is indeed the case. Exposure of NPE cells to 100 nM bafilomycin A₁ for 10 min produced a significant increase in cell sodium content (Fig.
6, A and B). The sodium increase was abolished when cells received baflomycin A1 in the presence of DMA. In the presence of baflomycin A1, the addition of ANG II did not cause a further detectable increase of cell sodium content (Fig. 6B).

**Fig. 5.** A: typical responses showing the influence of ANG II and baflomycin A1 on the pH response to a mixture of epinephrine (1 μM) + acetylcholine (10 μM). After a stable pH baseline was established, some cells were exposed to epinephrine + acetylcholine. Other cells were exposed to epinephrine + acetylcholine in the presence of either 100 nM baflomycin A1 (BAF) or 10 nM ANG II. Mean changes of pH are shown in B. Data are means ± SE from 4 different experiments. *Significant difference from the pH change observed in the epinephrine + acetylcholine group (P < 0.01). C: influence of ANG II on pH in the presence of BAF. The result of a typical experiment is shown. After a stable pH was obtained, the cells were exposed to 100 nM BAF for 5 min (left arrow), and then the superfusate was switched to contain 10 nM ANG II in the continued presence of 100 nM BAF (right arrow).

**Fig. 6.** Influence of ANG II and BAF on cytoplasmic sodium content in the presence or absence of DMA. Different groups of cells were exposed for 10 min to different agents: control (no BAF, DMA, or ANG II), BAF (100 nM), DMA (10 μM), ANG II (10 nM), ANG II + DMA (10 nM ANG II + 10 μM DMA), and BAF + DMA (100 nM BAF + 10 μM DMA) (A), or BAF + ANG II (100 nM BAF + 10 nM ANG II) (B). The cells were then washed with ice-cold isotonic MgCl2 and lysed with nitric acid, and the sodium content was measured by atomic absorption spectrophotometry. Data are means ± SE (n = 6). *Significant difference (P < 0.01) from the sodium content measured in control cells.

**H⁺-ATPase activity.** ATP hydrolysis measurements were conducted as a more direct way to examine the potential influence of ANG II on H⁺-ATPase activity. In control cells, H⁺-ATPase activity was 120.9 ± 19.2 pmol ATP hydrolyzed·mg protein⁻¹·min⁻¹ (means ± SE, n = 12). H⁺-ATPase activity was reduced by >50% in cells that had been exposed to 10 nM ANG II for 10
inclusion of EDTA. and calcium-activated ATPases were inhibited by the inclusion of sodium azide in the assay solution, ouabain and the omission of potassium from the assay activity was fully inhibited because of the addition of permeabilized by freeze-thaw, and H+/H1001–ATPase activity (BAF-insensitive ATPase activity) was 48.3 ± 9.6 pmol ATP hydrolyzed-mg protein−1min−1 in the control group and 59.9 ± 18.5 pmol ATP hydrolyzed-mg protein−1min−1 in the ANG II-treated group (no significant difference).

**DISCUSSION**

ANG II changes sodium dynamics in many tissues, and here we show that it causes an increase in the cytoplasmic sodium concentration in cultured rabbit NPE. The rate of Na+/K+–ATPase-mediated 86Rb transport increases by ~40% in NPE cells exposed to ANG II. A similar stimulatory effect of ANG II on Na+/K+–ATPase has been reported in astrocytes (20) and kidney (24). In cultured NPE, the mechanism responsible for the ANG II-induced stimulation of active Na+/K+ transport appears to involve the increase of cytoplasmic sodium concentration that occurs in ANG II-treated cells because prevention of the sodium increase abolished the stimulation of 86Rb uptake. The ability of saralasin and losartan but not PD-123319 to suppress the sodium increase suggests that the mechanism involves activation of AT1 receptors.

The ability of DMA to suppress both the rise of sodium and the stimulation of ouabain-sensitive 86Rb uptake in ANG II-treated cells suggests that the increase of Na+/K+–ATPase-mediated 86Rb transport is the result of an elevation in cytoplasmic sodium concentration. However, exposure of the cells to ANG II has been observed to cause an increase of cytoplasmic calcium concentration in cultured NPE (14), and an increase of cytoplasmic calcium concentration has been found to stimulate Na+/K+–ATPase-mediated active Na+/K+ transport in NPE and other tissues (19, 36). Elevated cytoplasmic calcium concentration has also been observed to cause activation of H+/H1001–ATPase in cultured NPE (13). However, there is no evidence to suggest that H+/H1001–ATPase activation occurs in ANG II-treated cells. Instead, the response to ANG II is consistent with H+/H1001–ATPase inhibition together with a concomitant activation of Na+/H+ exchange.

The increase in cytoplasmic sodium caused by ANG II was inhibited by DMA at a concentration of 10 μM, which is very close to the IC50 of 7 μM reported for inhibition of Na+/H+ exchange in adrenal glomerulosa cells by DMA (7). This suggests that stimulation of Na+/H+ exchange occurs in ANG II-treated cultured NPE. However, we cannot rule out the possibility that some sodium enters the ANG II-treated NPE via sodium channels because, although a 10 μM concentration of DMA is insufficient to inhibit sodium channels in lung airway epithelium (33), DMA at a higher concentration elicits detectable sodium channel blockade in cortical collecting duct cells (5). It should also be noted that the family of Na+/H+ exchangers contains six isoforms (22). The distribution of Na+/H+ exchanger isoforms can be quite different in different tissues (17, 22, 25). The sensitivity of different Na+/H+ exchanger isoforms to DMA varies considerably, and the IC50 for DMA can be much lower in some cell types (17, 25). The pattern of Na+/H+ exchanger isoforms expression is not known for the cultured rabbit NPE cells used in the present study. For cultured human NPE, Civan et al. (6) have demonstrated that the rate of the regulatory volume increase is inhibited by DMA at a concentration of 10 μM but not 1 μM.

The ability of ANG II to stimulate Na+/H+ exchange has been observed previously in vascular smooth muscle and other tissues (3, 7, 21, 26, 29). In a number of tissues, the stimulation of Na+/H+ exchange by ANG II is thought to involve activation of protein kinase C (11). Mechanistically, ANG II has been reported to stimulate Na+/H+ exchange partly as a result of a reduction in the Km for external sodium (7) and also through an increase of Vmax that may be, in part, the result of a rise in the number of Na+/H+ exchanger sites on the plasma membrane (3, 7). Although the observed increase of cytoplasmic sodium concentration suggests that Na+/H+ exchange-mediated proton export is stimulated in ANG II-treated NPE cells, no detectable increase of pH; was observed in cells exposed to ANG II.

We propose that ANG II inhibits H+/H1001–ATPase-mediated proton export in parallel with activation of Na+/H+ exchange. It was demonstrated earlier that when H+/H1001–ATPase is inhibited by baflomycin A1, activation of NaN+/H+ exchange occurs and pH; remains stable (12). Currently, we do not have sufficient information to determine whether a specific mechanism may exist to activate NaN+/H+ exchange when H+/H1001–ATPase-mediated proton export is inhibited.

The notion of H+/H1001–ATPase inhibition in ANG II-treated cells is supported by the finding that ANG II

![Graph](http://ajpcell.physiology.org/)

Fig. 7. Influence of ANG II on H+/H1001–ATPase activity. Cells were incubated for 10 min in the presence or absence of 10 nM ANG II. The ANG II-containing solution was then removed, the cells were permeabilized by freeze-thaw, and H+/H1001–ATPase activity (BAF-sensitive ATP hydrolysis) was measured. Data are means ± SE (n = 12). *Significant difference from control (P < 0.05). Basal ATPase activity (BAF-insensitive ATPase activity) was 46.3 ± 9.6 pmol ATP hydrolyzed-mg protein−1min−1 in the control group and 58.9 ± 18.5 pmol ATP hydrolyzed-mg protein−1min−1 in the ANG II-treated group (no significant difference).
exposure resulted in a significant reduction of pH₃ in cells in which Na⁺/H⁺ exchange was inhibited by DMA. ANG II and the H⁺-ATPase inhibition baflomycin A₁ exert a similar influence on pH₃, lowering pH in DMA-treated cells but not in the absence of DMA. Baflomycin A₁ is a highly selective inhibitor of V-type ATPase activity (4) and is not known to inhibit Na⁺/K⁺-ATPase or calcium ATPase activity. Baflomycin A₁ and ANG II have a similar effect on cytoplasmic sodium, both causing a sodium increase that is prevented by DMA. Importantly, ANG II failed to cause an additional increase of sodium in cells exposed to the H⁺-ATPase inhibitor baflomycin A₁. Furthermore, ANG II and baflomycin A₁ act similarly to suppress the cytoplasmic alkalization responses that occur after either depolarization with potassium-rich solution or exposure to a mixture of acetylcholine and epinephrine.

Taken together, the cytoplasmic sodium and pH responses constitute indirect evidence for H⁺-ATPase inhibition in ANG II-treated cells. More direct evidence in support of this concept comes from the results of ATP hydrolysis measurements made by using cultured NPE cells permeabilized by freeze-thaw; a 10-min episode of pretreatment with ANG II was observed to cause significant reduction of H⁺-ATPase activity. ANG II-mediated H⁺-ATPase inhibition has also been proposed in studies of kidney cortical collecting duct segments where ATP hydrolysis was measured (18, 30). However, not all tissues respond in the same way. ANG II stimulates H⁺-ATPase activity in proximal tubule (32).

The detection of ANG-converting enzyme and prorenin at the basal region of the NPE has led previous investigators to suggest that ANG II might be involved in the regulation of aqueous humor production (27, 28). Investigator to suggest that ANG II might be involved in the regulation of aqueous humor production (27, 28).

REFERENCES


