Endothelin-Induced Increased Aldosterone Activity Mediates Augmented Distal Nephron Acidification as a Result of Dietary Protein

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The hypothesis that increased dietary protein augments distal nephron acidification through endothelin-mediated increased aldosterone activity was tested. Munich-Wistar rats were studied after 3 wk of diets with 50% high protein (HiPro) and 20% control (CON) casein-provided protein, the latter comparable to standard diet. HiPro *versus* CON rats had higher distal nephron H^+ secretion by *in vivo* microperfusion as shown previously. Perfusion with inhibitors of Na^+/H^+ exchange (EIPA, 10^{-5} M), H^+-ATPase (bafilomycin, 10^{-7} M), and H^+-K^+-ATPase (Sch 28080 [10^{-5} M] and ouabain [10^{-3} M]) support that higher Na^+/H^+ exchange and higher H^+-ATPase but not higher H^+-K^+-ATPase activity mediated increased H^+ secretion in HiPro rats. Oral bosentan, an endothelin A/B receptor antagonist, decreased distal nephron H^+ secretion in HiPro rats as a result of reduced Na^+/H^+ exchange and H^+-ATPase activity as shown previously by the authors' laboratory. HiPro *versus* CON rats had higher plasma aldosterone (60.9 ± 5.9 *versus* 42.2 ± 4.4 pg/ml; $P < 0.024$) and higher urine aldosterone excretion (21.9 ± 3.9 *versus* 10.5 ± 2.8 ng/d; $P < 0.04$) in the absence but not presence of bosentan, consistent with endothelin-mediated increased aldosterone secretion. HiPro rats that did *versus* did not ingest the aldosterone antagonist spironolactone had lower distal nephron H^+ secretion (29.2 ± 3.3 *versus* 42.1 ± 3.8 pmol/mm per min; $P < 0.05$) as a result of lower H^+-ATPase activity without differences in Na^+/H^+ exchange or H^+-K^+-ATPase activity. The data support that dietary protein provided as casein increases distal nephron acidification through endothelin-stimulated Na^+/H^+ exchange and endothelin-stimulated aldosterone secretion that increases H^+-ATPase activity.

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Dietary acid as ammonium (NH_4^+) salts augments distal nephron acidification (1,2) through endothelin-dependent mechanisms (3). Dietary intake of acid-producing protein also increases distal nephron acidification (4,5) by mechanisms that are also endothelin dependent (5). In the latter studies, augmented distal nephron acidification was due to an endothelin-mediated increase in both Na^+/H^+ exchange and H^+-ATPase activity (5). Endothelin stimulates Na^+/H^+ exchange *in vitro* (6–8), but no studies report that endothelin increases *in vitro* H^+-ATPase activity. Consequently, endothelin might stimulate H^+-ATPase activity indirectly given the many endocrine alterations induced by a systemic acid challenge, including stimulation of the renin-angiotensin-aldosterone axis (9,10). Because increased dietary protein increases serum aldosterone (11), aldosterone increases distal nephron H^+-ATPase activity (12), and endothelin infusion increases aldosterone secretion *in vivo* (13,14), the present studies tested the hypothesis that the endothelin-mediated increase in distal nephron H^+-ATPase activity induced by acid-producing dietary protein is due to increased aldosterone activity.

Materials and Methods

Animals and Diet Protocol

Male and female Munich-Wistar rats (Harlan Sprague-Dawley, Houston, TX; 200 to 220 g) ate standard rat chow (Prolab RMH 2500 with 23% protein) for 1 wk, then ate a custom minimum electrolyte diet with protein as purified high-nitrogen casein (ICN Nutritional Biochemicals, Cleveland, OH) for 3 wk. High protein (HiPro) and control (CON) rats ate 50 and 20% protein, respectively. In preliminary studies, similar-weight rats ate 24.6 ± 0.9 and 27.1 ± 1.2 g/d, respectively ($n = 4$, $P = 0.15$), so all rats received 24 g/d diet to ensure similar and complete intake. Some ingested bosentan (Actelion, Allschwil, Switzerland), a nonpeptide endothelin A/B receptor antagonist (15), mixed with study diet (100 mg/kg body wt per d). This oral dose blocks action of pressor doses of intravenous big endothelin-1 (ET-1) for >24 h (15). Others ingested spironolactone (400 mg/kg body wt per d) dissolved in olive oil and mixed with diet. This oral dose is in excess of that required for virtually complete mineralocorticoid receptor blockade *in vivo* (16). All drank distilled H_2O *ad libitum*.

Urine Net Acid and ET-1 Excretion

We measured daily urine net acid excretion (NAE) (17) and ET-1 (3) in a 24-h sample collected as described (5) from eight each of HiPro and CON rats in metabolic cages. We examined the effect of endothelin and aldosterone receptor blockade with bosentan and spironolactone, respectively, on urine NAE in paired and separate groups of eight each

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(four with and four without drug) of HiPro and CON rats. NAE was the mean for each animal group.

Arterial Blood Parameters

We measured pH, P_{CO_2}, calculated [HCO_3] (IRMA Blood Analysis System, Diametrics Medical, Inc., St. Paul, MN) and total CO_2 (TCO_2) by ultrafluorometry (see below) in 1.0 ml of blood from a chronic carotid arterial catheter in eight each of awake, gently restrained, and calm HiPro and CON rats to assess the effects of HiPro on plasma acid-base parameters. We also measured arterial BP through this chronic arterial catheter as done previously (18).

Micropuncture Protocol

Animals were prepared for micropuncture of accessible distal tubules (19) at weeks 1 and 3. *In situ* early distal flow rate for HiPro and CON rats was 9.4 ± 0.7 (n = 6) and 6.4 ± 0.4 nl/min (n = 8), respectively. Separate superficial distal nephrons of HiPro and CON rats were each perfused 9 and 6 nl/min with a Hampel pump to approximate respective *in situ* flow rates. We measured distal tubule transepithelial potential difference to calculate blood-to-lumen HCO_3 permeability (19). After the micropunctured (left) kidney was weighed, perfused nephron length was determined by measuring the length of a latex cast injected after micropuncture, recovered after acid digestion of the kidney (19). We measured [HCO_3] in stellate vessel plasma to determine peritubular blood-to-lumen HCO_3 gradient for calculating transepithelial H^+/HCO_3 passive permeability (19). Diet but not H_2O was withheld the evening before micropuncture to yield higher baseline HCO_3 reabsorption (20), as done previously (19).

The perfusion solutions used are shown in Table 1. Solution 1 contained 5 mM HCO_3 and 40 mM Cl^- to approximate their concentrations at the early distal nephron *in situ* (21). Solution 2 contained Cl^- but no HCO_3 to measure Cl^--dependent luminal HCO_3 accumulation and to calculate an "apparent" blood-to-lumen H^+/HCO_3 permeability (19). Solution 3 was HCO_3^-- and Cl^--free and contained 0.5 mM acetazolamide to inhibit transtubule H^+/HCO_3 transport and was used to determine "passive" blood-to-lumen H^+/HCO_3 permeability (19). We used this passive permeability determined using solution 3 to calculate passive blood-to-lumen HCO_3 secretion when perfusing with the HCO_3-containing solution 1 (19). We used the apparent blood-to-lumen H^+/HCO_3 permeability determined from perfusing with solution 2 to calculate "total" HCO_3 secretion when perfusing with HCO_3-containing solution 1 (19). We subtracted calculated passive HCO_3 secretion from calculated total HCO_3 secretion to obtain "net" HCO_3 secretion when perfusing with solution 1 (19). The HCO_3 secretion reported herein is the net HCO_3 secretion that excludes the passive HCO_3 secretion calculated as described above. Distal nephron H^+ secretion was calculated by subtracting the calculated net HCO_3 secretion (a negative value) from the measured net HCO_3 reabsorption (HCO_3 perfused into the distal nephron minus HCO_3 collected) (18). All perfusing solutions contained raffinose to minimize fluid transport and gluconate substituted for Cl^- when necessary (19). Each surface distal nephron was perfused with each perfusing solution in the following order: 1, 2, 3. Previous studies showed that the order of perfusing solutions did not affect calculations of the components of distal nephron HCO_3 reabsorption (19).

Table 1. Perfusate composition (mM)

	Solution 1	Solution 2	Solution 3
Na^+	61	61	61
K^+	4	4	4
Cl^-	40	40	0
HCO_3^-	5	0	0
Gluconate	20	25	65
Acetazolamide	0	0	0.5
Raffinose	200	200	200

Identification of H^+ Transport Mediators of HiPro-Induced Changes in Distal Nephron Acidification

We compared the net decrease in distal nephron H^+ secretion in response to specific H^+ transport inhibitors to determine contributions of individual distal nephron H^+ transporters to differences in overall distal nephron H^+ secretion in HiPro *versus* CON rats. The contribution of Na^+/H^+ exchange and H^+-ATPase was determined by perfusing with solutions that contained EIPA (10^{-5} M) and bafilomycin (10^{-7} M), respectively, as done previously in our laboratory (22). Because the rat renal cortex contains the gastric isoform of H^+-K^+-ATPase (23), we perfused tubules with Sch 28080 (10^{-5} M) to inhibit this transporter (24) and determine whether its activity was increased in HiPro rats. Although the colonic isoform of H^+-K^+-ATPase has not been identified in the rat renal cortex (25) and its presence has not been induced by dietary NH_4Cl (25), we additionally perfused distal nephrons with solutions that contained both Sch 28080 (10^{-5} M) and ouabain (10^{-3} M), the latter inhibiting the colonic H^+-K^+-ATPase isoform (24). The latter perfusion was done to determine whether HiPro induced and/or increased activity of either the gastric or colonic H^+-K^+-ATPase isoforms. Greater inhibitor-induced decrease in H^+ secretion in HiPro *versus* CON rats determined increased activity of the H^+ transporter inhibited by that compound (22).

Analytical Methods

Immediately after experiment termination, initial and collected perfusate and stellate vessel plasma samples were analyzed for inulin (19) and for TCO_2 using flow-through ultrafluorometry (26) as described previously (27). All tubule fluid and plasma TCO_2 were measured on the experimental day by comparing fluorescence of a 7- to 8-nl sample aliquot (corrected for a distilled H_2O blank run with each sample group) with a standard curve as described previously (27). This technique actually measures TCO_2, but we refer to this measured value as HCO_3 for simplicity.

Statistical Analyses

Data were expressed as means ± SEM. One to two distal nephron segments were perfused per animal. When two tubules were perfused in an animal, results were meaned to yield a single value for that animal. Group number is number of animals. Paired perfusions of the same tubule were compared using paired t test; otherwise, ANOVA was used for multiple group comparisons. We used the Bonferroni method for multiple comparisons ($P < 0.05$) of the same parameter among groups.

Results

Effect of HiPro and Receptor Antagonists on Animal/Kidney/Tubule Growth

The HiPro protocol increases body and kidney weight as well as the length of the accessible distal tubule (5). Neither the endothelin A/B receptor antagonist bosentan nor the mineralocorticoid antagonist spironolactone affected animal/kidney weight, tubule length, food intake, or urine output in either group.

Effect of HiPro on Arterial Acid-Base Parameters of Conscious Animals

Table 2 shows that HiPro compared with CON arterial pH, Pco_2, and calculated [HCO_3] by blood gases were not different but plasma TCO_2 by ultrafluorometry was lower in HiPro. Mean BP (MBP) was not different in HiPro and CON rats (111.5 ± 2.4 *versus* 108.3 ± 2.1 mmHg; $P = 0.33$; $n = 8$ animals each).

Effect of HiPro on Renal Acidification

HiPro rats have higher urine NAE than CON rats mediated by higher urine NH_4^+ and lower HCO_3 excretion with similar excretion of titratable acid (5). Distal nephron net HCO_3 reabsorption was higher in HiPro than CON rats perfused at 6 and 9 nl/min as a result of higher H^+ secretion and to less extent to lower HCO_3 secretion (5).

Transport Mediators of Enhanced Distal Nephron Acidification in HiPro Rats

Figure 1 shows greater net decrease in distal nephron H^+ secretion in HiPro than in CON rats with EIPA (−15.5 ± 1.0 *versus* −10.5 ± 0.9 pmol/mm per min [$P < 0.003$ at 6 nl/min, $n = 8$ animals each] and −15.9 ± 1.1 *versus* −9.8 ± 0.9 pmol/mm per min [$P < 0.001$ at 9 nl/min, $n = 8$ animals each]) and bafilomycin (−14.5 ± 1.1 *versus* −7.7 ± 0.6 pmol/mm per min [$P < 0.001$ at 6 nl/min, $n = 8$ animals each] and −12.4 ± 1.0 *versus* −6.1 ± 0.9 pmol/mm per min [$P < 0.001$ at 9 nl/min, $n = 8$ animals each]), consistent with enhanced Na^+/H^+ exchange and increased H^+-ATPase activity, respectively. Neither Sch 28080-induced (−3.4 ± 0.9 *versus* −2.8 ± 0.8 pmol/mm per min [$P = 0.52$ at 6 nl/min, $n = 8$ animals each] and −4.0 ± 0.8 *versus* −3.3 ± 0.7 pmol/mm per min [$P = 0.29$ for 9 nl/min, $n = 8$ animals each]) or Sch 28080+ouabain-induced (−3.1 ± 1.0 *versus* −1.8 ± 0.9 pmol/mm per min [$P = 0.37$ at 6 nl/min, $n = 4$ animals each] and −3.5 ± 0.9 *versus* −2.7 ± 0.8 pmol/mm per min [$P = 0.53$ for 9 nl/min, $n = 4$ animals each]) net decrease in H^+ secretion differed in HiPro and CON rats, consistent with no increased H^+-K^+-ATPase activity in HiPro rats.

Effect of HiPro on Renal ET-1 and Aldosterone Production

HiPro increases urine ET-1 excretion, ET-1 addition to renal cortical microdialysate, and renal cortical mRNA (5). Figure 2 shows that HiPro compared with CON rats had higher plasma aldosterone (60.9 ± 5.9 *versus* 42.2 ± 2.4 pg/ml; $P = 0.033$, ANOVA; $n = 8$ animals each) and higher urine aldosterone excretion (21.9 ± 3.5 *versus* 10.5 ± 1.8 ng/d; $P = 0.035$, ANOVA; $n = 8$ animals each) in the absence of bosentan. By contrast, plasma aldosterone (51.4 ± 4.9; $P = 0.34$ *versus* CON; $n = 8$ animals) and urine aldosterone excretion (13.0 ± 2.7; $P = 0.45$ *versus* CON; $n = 8$ animals) were not different between bosentan-ingesting HiPro and CON rats, respectively.

Effect of Endothelin and Mineralocorticoid Receptor Blockade on Arterial Blood and Urine Parameters

Bosentan did not affect CON arterial plasma acid-base parameters (data not shown). Plasma TCO_2, urine net NAE, and MBP were not different in HiPro rats that did compared with did not ingest bosentan (5). By contrast, CON rats that ingested spironolactone had lower plasma TCO_2 than concurrently studied CON rats that did not ingest spironolactone (22.9 ± 0.6 *versus* 25.2 ± 0.6 mM; $P = 0.017$; $n = 8$ animals each) but similar urine NAE (3569 ± 448 *versus* 4460 ± 639 μM/d; $P = 0.27$; $n = 8$ animals each) and MBP (107.8 ± 2.2 *versus* 110.1 ± 2.2 mmHg; $P = 0.74$; $n = 8$ animals each). Similarly, HiPro rats that did compared with concurrently studied HiPro rats that did not ingest spironolactone had lower plasma TCO_2 (20.2 ± 0.8 *versus* 23.0 ± 0.5 mM; $P = 0.02$; $n = 8$ animals each) but similar urine NAE (6210 ± 589 *versus* 7067 ± 937 μM/d; $P = 0.45$; $n = 8$ animals each) and MBP (110.7 ± 2.2 *versus* 111.5 ± 2.4 mmHg; $P = 0.81$; $n = 8$ animals each).

Effect of Endothelin and Mineralocorticoid Receptor Blockade on HiPro-Induced Augmented Distal Nephron Acidification

Distal nephron acidification was not different between CON rats that did and did not receive bosentan (5). Similarly, distal nephron net HCO_3 reabsorption was not different between CON rats that did and did not receive spironolactone whether perfused at 6 nl/min (10.8 ± 1.7 *versus* 12.9 ± 1.4 pmol/mm per min, respectively; $P = 0.36$; $n = 8$ animals each) or 9 nl/min (12.8 ± 1.9 *versus* 14.9 ± 1.4 pmol/mm per min; $P = 0.39$; $n = 8$ animals each). By contrast, HiPro rats that did compared with did not receive bosentan had lower distal tubule net HCO_3 reabsorption that was mediated by lower H^+ secretion and higher HCO_3 secretion (5). Similarly, Figure 3 shows that HiPro rats that did compared with did not receive spironolactone had lower distal nephron net HCO_3 reabsorption (26.7 ± 3.0 *versus* 39.0 ± 3.1 pmol/mm per min; $P < 0.013$; $n = 8$ animals each). Like the decreased distal nephron net HCO_3 reabsorption as a result of bosentan, the decreased distal nephron net HCO_3 reabsorption as a result of spironolactone was mediated by reduced H^+ secretion (29.2 ± 3.3 *versus* 42.1 ± 3.8 pmol/mm per min; $P < 0.023$; $n = 8$ animals each). Unlike decreased distal nephron net HCO_3 reabsorption induced by bosentan that was also mediated by increased distal nephron HCO_3 secretion (5),

Table 2. Plasma acid-base data in conscious animals after 3 weeks of dietary protein[a]

	pH	Pco_2 (mmHg)	Calculated [HCO_3] (mM)	Measured [TCO_2] (mM)
CON (20% protein; $n = 8$)	7.41 ± 0.02	39.2 ± 1.0	24.1 ± 0.9	25.2 ± 0.6
HiPro (50% protein; $n = 8$)	7.38 ± 0.02	38.5 ± 1.0	22.1 ± 0.8	23.3 ± 0.5[b]

[a]Values are means ± SEM. CON, control; HiPro, 3 wk of 50% dietary protein; TCO_2, total CO_2.
[b]$P < 0.05$ *versus* 20% protein.

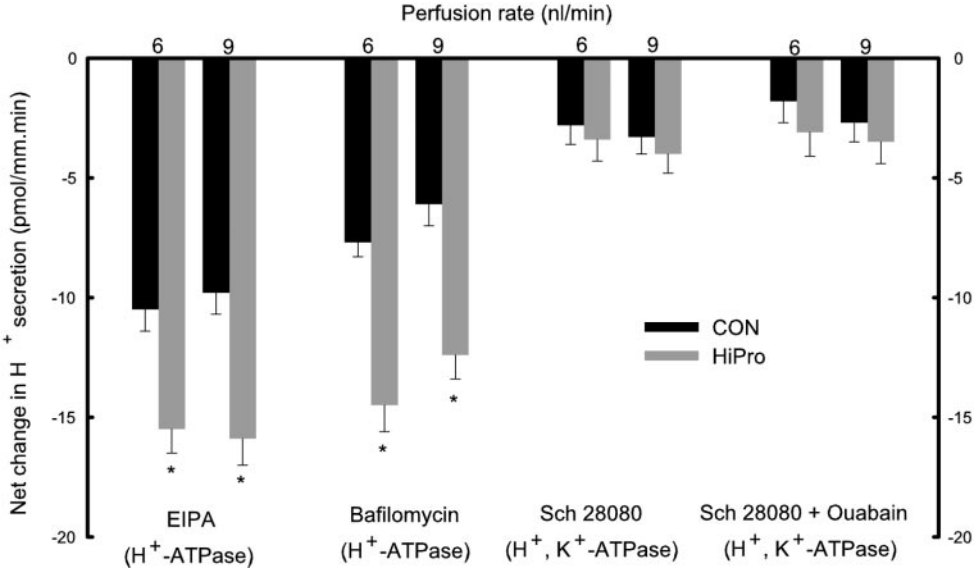

Figure 1. Net change in distal nephron proton (H^+) secretion in response to *in vivo* microperfusion at 6 or 9 nl/min with inhibitors of Na^+/H^+ exchange (EIPA), H^+-ATPase (bafilomycin), gastric H^+-K^+-ATPase (Sch 28080), and gastric + colonic H^+-K^+-ATPase (Sch 28080 + ouabain). *$P < 0.05$ *versus* control (CON).

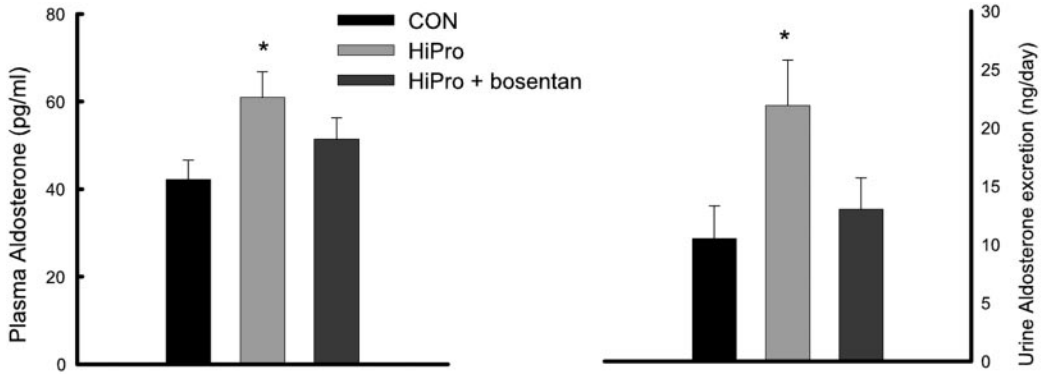

Figure 2. Plasma aldosterone (left) and urine aldosterone excretion (right) in CON, HiPro, and HiPro animals that received the endothelin A/B receptor antagonist bosentan. *$P < 0.05$ *versus* CON.

this component of distal nephron net HCO_3 reabsorption was not different in HiPro rats that did compared with did not ingest spironolactone (-5.4 ± 0.6 *versus* -3.9 ± 0.7 pmol/mm per min; $P = 0.13$; $n = 8$ animals each).

Effect of Endothelin and Mineralocorticoid Receptor Blockade on Enhanced H^+ Transporter Activity Induced by HiPro

Net decrease in distal nephron H^+ secretion was not different in bosentan-ingesting compared with noningesting HiPro rats that were perfused at 9 nl/min with EIPA and bafilomycin, consistent with no additional effect of these inhibitors on Na^+/H^+ exchange and H^+-ATPase activity, respectively, in HiPro rats with endothelin A/B receptor blockade (5). There was also no difference in the net decrease in H^+ secretion in bosentan-ingesting compared with noningesting HiPro rats that were perfused with Sch 28080 (5), consistent with no effect of endothelin A/B receptor blockade on H^+-K^+-ATPase activity in HiPro rats. By contrast, Figure 4 shows that spironolactone-ingesting compared with noningesting HiPro rats had less bafilomycin-induced decrease in distal nephron H^+ secretion when perfused at 9 nl/min (-6.5 ± 1.2 *versus* -14.5 ± 1.1 pmol/mm per min; $P < 0.001$; $n = 8$ animals each), consistent with spironolactone-induced inhibition of the previously demonstrated augmented H^+-ATPase activity of HiPro rats. By contrast, EIPA-induced (-13.2 ± 1.4 *versus* -15.9 ± 1.1 pmol/mm per min; $P = 0.15$ at 9 nl/min; $n = 8$ animals each), Sch 28080-induced (-6.1 ± 0.9 *versus* -4.0 ± 0.8 pmol/mm per min; $P = 0.10$ at 9 nl/min; $n = 8$ animals each), and Sch 28080 +ouabain-induced (-8.9 ± 1.6 *versus* -6.6 ± 0.9 pmol/mm per min; $P = 0.26$ at 9 nl/min; $n = 4$ animals each) decrease in distal nephron H^+ secretion was not different in spironolactone-ingesting compared with noningesting HiPro rats, consistent with no spironolactone effect on Na^+/H^+ exchange or H^+-K^+-ATPase activity, respectively, in HiPro rats.

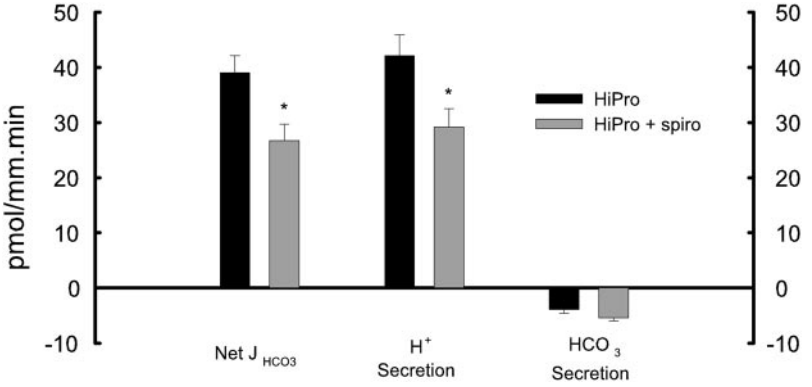

Figure 3. Distal tubule net HCO_3 reabsorption (Net J_{HCO3}) and its components HCO_3 and H^+ secretion by *in vivo* microperfusion in HiPro-noningesting and -ingesting the aldosterone receptor antagonist spironolactone. *$P < 0.05$ *versus* CON.

Discussion

These studies tested the hypothesis that the endothelin-dependent increase in distal nephron H^+-ATPase activity in HiPro rats (5) is mediated through endothelin-stimulated aldosterone secretion that enhances H^+-ATPase activity. HiPro as casein was associated with increases of plasma aldosterone and urine aldosterone excretion in the absence but not presence of the endothelin A/B receptor antagonist bosentan. Furthermore, reduction in distal nephron H^+ secretion induced by the H^+-ATPase inhibitor bafilomycin was less in the presence than absence of the aldosterone receptor antagonist spironolactone in HiPro rats. Together, the present studies support that HiPro as casein induces endothelin-stimulated secretion of aldosterone that increases distal nephron H^+-ATPase activity. Because increased dietary protein induces endothelin-mediated increased distal nephron Na^+/H^+ exchange (5), augmented H^+ secretion induced by HiPro is mediated by the combined effects of endothelin and aldosterone.

Increased intake of dietary protein that is composed of acid-producing amino acids increases metabolic acid production and renal acid excretion (28) that is mediated by augmented distal nephron acidification (4,5). Augmented distal nephron acidification is manifest by increased net HCO_3 reabsorption (29), consistent with increased H^+ secretion that reclaims filtered HCO_3 and promotes NH_4^+ secretion (30). The resulting decrease in HCO_3 delivery to the terminal nephron also promotes NH_4^+ secretion (30) and permits secreted H^+ to titrate non-HCO_3 buffers that effect net acid excretion rather than HCO_3 reclamation (31). Previous studies showed that the augmented distal nephron H^+ secretion induced by HiPro is due to increased Na^+/H^+ exchange and increased H^+-ATPase activity (5). The previous (5) and present studies support that this enhanced Na^+/H^+ exchange and H^+-ATPase activity is mediated by endothelin and aldosterone, respectively. Augmented distal nephron acidification induced by dietary acid is also due to decreased distal nephron HCO_3 secretion (2), again through an endothelin-dependent mechanism (3). Endothelin infusion decreases distal nephron HCO_3 secretion (32), but whether this effect is direct or is mediated through another agent was not determined. Consequently, endothelins play an important role in mediating both the increased H^+ secretion and decreased HCO_3 secretion induced by dietary acid that mediate the increased distal nephron acidification in this setting.

Spironolactone was used as the aldosterone antagonist in the present studies to demonstrate the aldosterone-mediated increase in distal nephron H^+-ATPase activity. Other investigators re-

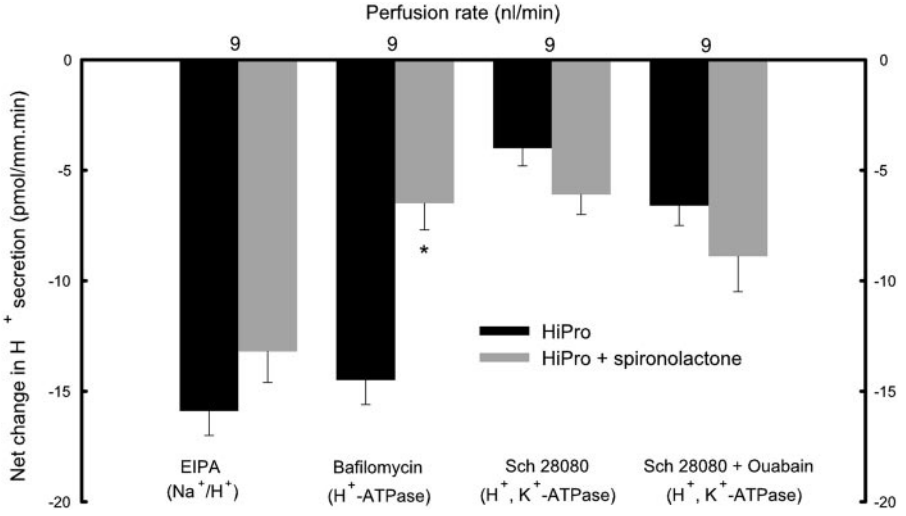

Figure 4. Net change in distal nephron proton (H^+) secretion in HiPro response to *in vivo* microperfusion at 9 nl/min with inhibitors of Na^+/H^+ exchange (EIPA), H^+-ATPase (bafilomycin), and H^+-K^+-ATPase (Sch 28080). *$P < 0.05$ *versus* HiPro.

ported studies supporting that spironolactone increases endogenous acid production (33) that might have contributed to the decrease in plasma TCO_2 observed in both HiPro and CON animals that ingested spironolactone. The microperfusion studies reported in the present studies nevertheless support that aldosterone-mediated increased H^+-ATPase activity contributed to the augmented H^+ secretion of HiPro animals.

The mechanisms by which dietary acid as NH_4^+ salts (3) or as acid-producing dietary protein (4,5) increases renal endothelin production and cell type that is the endothelin source are not clear. Renal microvascular endothelial cells that are exposed to an acid extracellular environment *in vivo* increase ET-1 release into the culture medium (34), possibly contributing to increased renal interstitial fluid ET-1 levels in response to dietary acid (3). Nevertheless, renal epithelial cells also make and secrete ET-1 (35) and might also be a source of the augmented renal endothelin. The present studies support that the increased plasma and urine aldosterone associated with HiPro was mediated by endothelin. Similarly, chronic dietary NH_4Cl increases plasma aldosterone (36), and endothelin might also mediate the increased plasma aldosterone that occurs in this setting. Endothelin infusion increases aldosterone secretion in rats *in vivo* (13,14), and endothelin is an important paracrine regulator of zona glomerulosa function (37).

In summary, HiPro as purified casein augments distal nephron H^+ secretion through aldosterone-stimulated increased H^+-ATPase activity. The increased aldosterone in this setting is mediated through endothelin, which itself increases distal nephron Na^+/H^+ exchange. The data support that aldosterone and endothelins are important mediators of the increased distal nephron acidification associated with increased intake of dietary protein, the common dietary challenge to systemic acid-base homeostasis faced by humans.

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