

Prostaglandins Inhibit Renal Ammoniogenesis in the Rat

Edward R. Jones, Thomas R. Beck, Shiv Kapoor, Robert Shay, and Robert G. Narins

Departments of Medicine and Physiology, Section of Nephrology, Temple University Health Sciences Center, Philadelphia, Pennsylvania 19140

Abstract. We describe the inhibitory effect of prostaglandins (PGs) on in vivo rat renal ammonia synthesis. The influence of systemic pH upon urinary PG excretion and ammoniogenesis was also investigated. Finally, PG production by incubated rat renal cortical slices was suppressed to investigate the PG-ammonia interplay in the absence of changes in renal blood flow, glomerular filtration rate, ambient electrolyte concentrations or extrarenal hormonal factors.

In vivo ammonia synthesis doubled and PG excretion fell by 44% in normal rats, after intravenous administration of 1 mg/kg of meclofenamate. Higher doses of meclofenamate further augmented ammonia production and further reduced PG excretion. PG depletion was also associated with an increase in fractional excretion of ammonia (FE_{NH_3}) that was independent of changes in urine flow rate or pH.

Acute metabolic acidosis (AMA) increased total ammonia synthesis but also stimulated PG production. Administration of meclofenamate to rats with mild AMA markedly reduced urinary PG excretion, further augmented ammonia synthesis, and significantly increased the FE_{NH_3} . Inhibition of stimulated PG synthesis during severe AMA did not increase ammoniogenesis or FE_{NH_3} . Acute metabolic alkalosis did not alter production of PGs or ammonia, but reduced the FE_{NH_3} by

42%. Meclofenamate nearly normalized the FE_{NH_3} but stimulated synthesis to a lesser degree than was seen in nonalkalotic rats that received meclofenamate.

Inhibition of PG synthesis in incubated rat renal cortical slices also stimulated ammoniogenesis. Conversely, stimulation of PG synthesis decreased ammonia production and acidification of the incubation medium increased prostaglandin $F_{2\alpha}$ production. Thus, in vitro findings support the in vivo results.

We conclude that PGs inhibit ammonia synthesis in normal rats and in those undergoing mild AMA. Severe acidosis overrides this inhibitory effect of PGs, whereas metabolic alkalosis suppresses the stimulatory effect of PG synthesis inhibition.

Introduction

Prostaglandins (PGs)¹ modulate many vital renal functions. Their effects on renal blood flow (RBF), glomerular filtration rate (GFR), water metabolism, and sodium handling have been extensively studied and recently reviewed (1). PGs appear to function primarily as negative-feedback inhibitors, i.e., the renal response elicited by various physiologic and pathologic stimuli appears to be the net result of a positive direct tissue effect of the stimulus plus the negative indirect inhibitory action of stimulated PG synthesis. These interactions are best illustrated by the effect of antidiuretic hormone (ADH) on renal water reabsorption. ADH enhances water reabsorption in the distal nephron and toad urinary bladder, but concomitantly stimulates PG synthesis, which in turn, reduces the hydro-osmotic effect of the hormone (2, 3). Prevention of ADH-stimulated PG synthesis enhances water reabsorption by unmasking the full, unopposed effect of the hormone (3).

Although a number of physiologic perturbations are known to influence renal ammoniogenesis (4), the effects of PGs on

A preliminary abstract was presented at the Annual Meeting of The American Federation for Clinical Research, 1983 (1983. *Clin. Res.* 31:431A) and reported at the National Meetings of The American Society of Nephrology, 1982.

Dr. Jones is a recipient of a Clinical Investigator Award of the National Institutes of Health.

Received for publication 19 December 1983 and in revised form 8 May 1984.

J. Clin. Invest.

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0021-9738/84/09/0992/11 \$1.00

Volume 74, September 1984, 992-1002

1. *Abbreviations used in this paper:* ADH, antidiuretic hormone; AMA, acute metabolic acidosis; FE_{NH_3} , fractional excretion of NH_3 ; GFR, glomerular filtration rate; KHB, Krebs-Henseleit bicarbonate buffer; mAMA, mild AMA; PG, prostaglandin; PGE_2 and $PGF_{2\alpha}$, prostaglandins E_2 and $F_{2\alpha}$; RBF, renal blood flow; sAMA, severe AMA.

this key urinary buffer have not been previously investigated. Many studies have demonstrated that acidification of the serosal medium of the toad bladder inhibits the action of ADH (5–9). Recently, Forrest et al. (9) confirmed these results and demonstrated that acidification of the medium is also associated with increased prostaglandin E₂ (PGE₂) production. The addition of meclofenamate or indomethacin, inhibitors of PG production, to acidified bladders restored the effect of ADH. In vivo studies by Beck and Kim (10) demonstrated that rats with acute metabolic acidosis (AMA) had a diminished response to physiologic amounts of ADH, i.e., free water reabsorption was diminished. Together, these studies clearly demonstrate a potentially important interplay between acidosis and renal PG synthesis.

The purpose of the present study was to define the influence of PGs on renal ammonia synthesis in vivo and in vitro under normal conditions and during various acid-base perturbations. We demonstrate that PGs are potent inhibitors of the renal synthesis of this important buffer. This conclusion is based on the following observations. Inhibition of PG synthesis markedly stimulated renal ammoniagenesis in normal rats. Mild acute metabolic acidosis (mAMA) simultaneously stimulated renal PG synthesis and ammoniagenesis. Inhibition of acidosis-induced PG production further enhanced ammonia generation. However, the inhibitory effect of PGs was completely overridden by severe metabolic acidosis (sAMA), while metabolic alkalosis dampened the stimulatory effect of inhibited PG synthesis. The physiologic and pathophysiologic implications of these findings are discussed.

Methods

Rat chow and drinking water were given *ad lib.* to male Sprague-Dawley rats (275–375 g) before study.

In vivo studies

Clearance methods (Fig. 1). Rats, anesthetized with intraperitoneal inactin (150 mg/kg) were tracheotomized and ventilated with a small animal respirator (Harvard Apparatus Co., Inc., S. Natick, MA), which maintained PCO₂ at 40±2 torr. Adjustments in tidal volume were only made during the equilibration period (Fig. 1). The jugular vein was catheterized with PE 50 tubing for infusion and a similar carotid artery catheter was connected to a Satham transducer for monitoring blood pressure and for sampling blood for acid-base parameters. A suprapubic cystostomy was performed and flared PE 50 tubing was inserted for collection of urine. After a midline incision, the abdomen was closed with staples to facilitate subsequent access to the renal vein. After preparative surgery, each rat received a bolus injection of normal saline equal to 1% of body weight. The saline contained 10 µCi of [³H]inulin, the extraction and clearance of which was used to measure RBF and GFR (11). A constant infusion (0.11 ml/min) of 0.45% saline that contained 2.5% mannitol and [³H]inulin (10 µCi/h) was maintained throughout the study, unless otherwise noted. A 70-min equilibration period was followed by a 40-min experimental period, during the last 10 min of which urine was collected into preweighed conical vials. At the end of the experiment, 1.5 ml of renal venous blood was slowly aspirated via a 27-gauge curved needle on a heparinized syringe; 2.0 ml of arterial blood was then aspirated (12).

Urine was analyzed for pH, total CO₂, ammonia, PGE₂ and prostaglandin F_{2α} (PGF_{2α}), and [³H]inulin. Hematocrit, pH, total CO₂, ammonia, and [³H]inulin were measured on arterial blood, while renal venous blood was only assayed for ammonia and [³H]inulin.

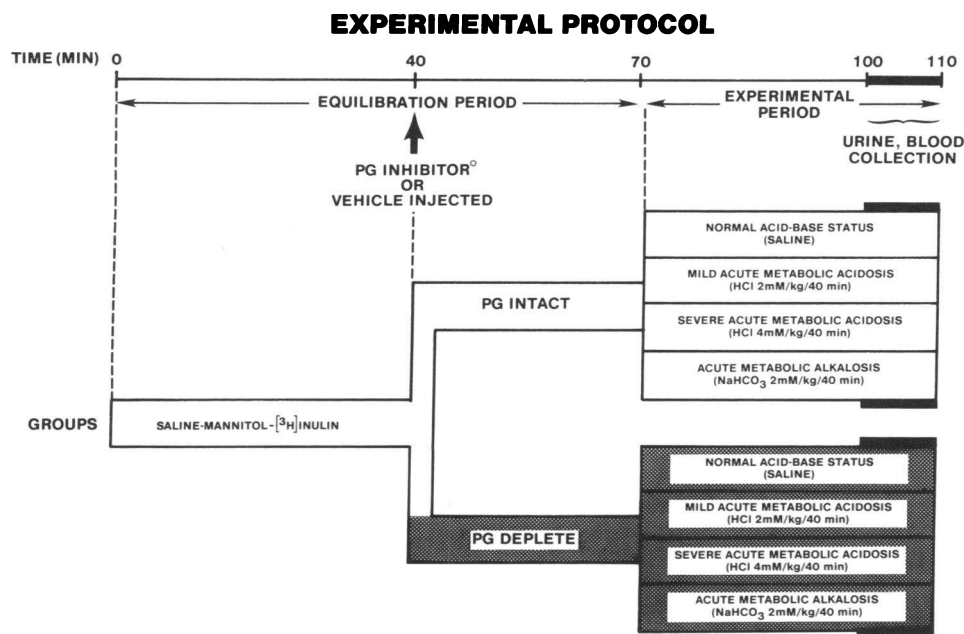


Figure 1. Outline of the protocol for the in vivo clearance studies performed in male Sprague-Dawley rats divided into PG INTACT (unhatched) and PG DEplete (hatched) groups. Meclofenamate (1 or 5 mg/kg), indomethacin (5 mg/kg), or their respective vehicles were administered at the indicated times. Each group was subdivided as noted. All infusions contained 2.5% mannitol and [³H]inulin and were delivered at a rate of 0.11 ml/min. In those subgroups undergoing acid-base perturbations, the saline was replaced isotonicly by HCl or NaHCO₃.

Alterations in PG synthesis

Preliminary experiments. Using anesthetized, ventilated rats, we determined the point at which maximal inhibition of PG synthesis occurred and the duration of this suppression. After collection of urine for 20 min, a single dose of 5 mg/kg of meclofenamate, dissolved in 0.15 M NaCl, was given intravenously. Urine was collected for five additional 20-min periods after the meclofenamate injection. PGs were then measured in all urine samples.

Meclofenamate administration (Fig. 1). Meclofenamate, 5 mg/kg body weight (high dose) or 1 mg/kg body weight (low dose), was given 60 min before urine and blood collection. As determined in preliminary experiments, 5 mg/kg resulted in unmeasurable PG excretion within 20 min of injection. Animals receiving meclofenamate were designated as "PG-deplete rats." Animals designated as "PG-intact rats," received the vehicle (saline) intravenously, 60 min before urine and blood collections.

Indomethacin administration. Additional studies were carried out with indomethacin, another cyclooxygenase inhibitor that differs structurally from meclofenamate. The drug, 5 mg/kg body weight, was solubilized in sodium carbonate and administered intravenously to normal rats in a fashion identical to that noted above. Control rats received vehicle alone.

Alteration in systemic pH

The acid-base perturbations described below were produced during the experimental period in PG-intact and -depleted rats.

Normal acid-base status. The mannitol-saline solution, initiated during the equilibration period, was continued at the same rate throughout the experimental period.

AMA. The mannitol-saline solution infused during the equilibration period was changed to a mannitol-hydrochloric acid solution that provided 2 mmol of acid/kg of body weight during the 40-min experimental period. These animals represent mild AMA. A second subgroup, which received 4 mmol of hydrochloric acid/kg body weight of hydrochloric acid during the experimental period, was designated severe AMA.

Acute metabolic alkalosis. The mannitol-saline solution was changed to 0.4 M sodium bicarbonate, which was infused during the 40-min experimental period and provided 2 mmol of alkali/kg body weight.

In vitro studies

Rat renal cortical slices were obtained as previously described (13). Slices taken from two rats were mixed and randomly placed in 25-ml Erlenmeyer flasks that contained 10 ml of Krebs-Henseleit bicarbonate buffer (KHB) containing (in millimolar): NaCl, 115; KCl, 5; MgSO₄, 1.2; CaCl₂, 1; NaHCO₃, 24; KH₂PO₄, 1.2; and glutamine, 2. Control flasks were glutamine-free. Media were separately designated: "PG intact": vehicle was added to the KHB; "PG inhibited": meclofenamate (20 µg/ml) was dissolved in water and added to KHB; in separate experiments, indomethacin (25 µg/ml) was added to 12 flasks that contained KHB. Equal volumes of diluent were added to the control flasks; "PG excess": Calcium ionophore A23187 (19 µM) was dissolved in ethanol and added to KHB to stimulate PG synthesis. Paired control flasks received the appropriate vehicle. The incubation media and slices were gassed with 5% CO₂:95% O₂, which resulted in a pH of 7.4. The flasks were placed in a Dubanoff metabolic shaker for 60 min at 37°C. Aliquots of the medium were obtained 5 min after initiation of incubation and again at 65 min and then analyzed for PGs and ammonia. Samples for ammonia were deproteinized with perchloric

acid while the remainder of the media was stored at -80°C until analyzed for PGs. The slices were desiccated at 110°C for 24 h, and then weighed. Acidosis (pH 7.0) was established by reducing the concentration of HCO₃⁻ to 12 mM while reciprocally increasing that of chloride.

Measurements

Ammonia. Plasma and urinary ammonia were determined by a modification of the method of Kurahasi et al. (14). The technique uses a Dowex (50 × 8 [200 mesh]) resin in the sodium form, which is placed in Pasteur pipettes that are plugged with glass wool. Plasma and urinary ammonia bind to the column and are then eluted with 4 M NaCl. Ammonia from eluted blood and urine and from precipitated KHB media was determined colorimetrically, by using a spectrophotometer (Perkin-Elmer Corp., Instrument Div., Norwalk, CT) (15). Recoveries of ammonia added to plasma, urine, and incubation media was 98 ± 3%. Measurements were performed in duplicate and the mean values were used for statistical analysis.

PGs. PGE₂ and PGF_{2α} were measured by radioimmunoassay by using standard techniques (16). Urine samples that contained tracer amounts of radioactive PGs for recoveries were acidified to pH 3.0–3.5 and extracted twice with 3 ml of ethylacetate. The organic phase from the extraction was evaporated to dryness and resuspended in ethylacetate/toluene. The samples were then purified on silicic acid columns. The PG fraction, eluted with toluene/ethylacetate/methanol, was evaporated and resuspended in buffer. PGE₂ and PGF_{2α} were then measured by radioimmunoassay. PGE₂ and PGF_{2α} were measured by radioimmunoassay on unextracted KHB. The antibody to PGE₂ was purchased from Sigma Chemical Co. (St. Louis, MO), while that to PGF_{2α} was a generous gift from Dr. M. Dunn, Case Western Reserve University (Cleveland, OH). The sensitivities of the antisera were: PGE₂:25 pg/ml; PGF_{2α}:50 pg/ml. Samples were run in triplicate and the final data were corrected for recovery. Recoveries ranged from 65 to 80% of the added tracer PGs.

Arterial and urine pH. A Radiometer pH meter (Copenhagen Radiometer pH Meter 27 with expanded scale) was used to anaerobically measure pH. Total CO₂ was determined manometrically with a Natelson microgasometer; bicarbonate and PCO₂ were calculated by using the Henderson-Hasselbalch equation. The pK' was corrected for temperature and the solubility coefficient, 0.0301 mmol CO₂/torr, was used.

GFR and RBF. The GFR was determined by standard calculations, by using [³H]inulin. The renal plasma flow was determined from the clearance of inulin corrected by the Wolf equation (17) and divided by the renal extraction of inulin (11). The RBF was calculated by dividing the RPF by [1 - hematocrit].

Calculations

Ammonia. In vivo total ammonia production was calculated as the product of the RBF and the renal venous, arterial plasma difference in ammonia concentration, plus the urinary excretion of ammonia. Production is expressed as nanomoles per minute per milliliter GFR. Fractional excretion of ammonia (FE_{NH₃}) refers to the fraction of renal ammonia synthesized that appears in the urine or {U_{NH₃}V ÷ [U_{NH₃}V + (RV_{NH₃} - A_{NH₃} × RBF)]} 100, where U is urine, V is volume, and RV_{NH₃} - A_{NH₃} is renal venous, arterial difference in NH₃ concentration.

In vitro production was calculated as the difference in ammonia accumulation over 60 min between slice-containing flasks with and without glutamine. The addition of ammonia to the incubation medium derives from the spontaneous non-tissue-mediated breakdown

of glutamine, from that carried into the medium with renal tissue, and finally from that generated by tissue from glutamine during the experimental period of incubation. Accordingly, the flask ammonia content at 5 min of incubation was subtracted from the value found at 65 min for flasks containing tissue slices with 2 mM glutamine and for those flasks with glutamine but without tissue. The ammonia contributed by spontaneous amino acid breakdown was subtracted. Ammonia synthesis was expressed as micromoles per gram of tissue dry weight per hour.

Prostaglandins. In vitro PG synthesis was determined by subtracting the media content of PG production at the end of 5 min of incubation from PGs produced after a 65-min incubation and expressed as picograms per gram dry weight per hour.

Statistical analyses. All results are expressed as the mean \pm 1 SE. When indicated, analysis of variance was performed to sort out intragroup significance. Statistical differences were determined by paired and unpaired *t* tests. Linear regression was determined by the method of least squares. Statistical comparisons of estimated parameters of the best-fit line were made by using the *t* test (18).

Medications and chemicals. Indomethacin (Sigma Chemical Co.), meclofenamate (Parke-Davis, Morris Plains, NJ), and calcium ionophore A23187 (Sigma Chemical Co.) were used in this study.

Results

In vivo studies

Preliminary studies. Within 20 min of the intravenous administration of 5 mg/kg of meclofenamate, urinary excretion of PGE₂ and PGF_{2 α} was unmeasurable (i.e., <20 pg/min). This extinction of PG synthesis was maintained for at least 100 min. Therefore, in studies of PG-depleted rats, meclofenamate administration preceded the experimental period by 30 min.

Dose-response effect of meclofenamate in normal rats. Hemodynamic and acid-base parameters in all groups are outlined in Tables I and II.

PG-intact rats. Total ammonia production was 281 \pm 19 nmol/min per ml GFR with 140 \pm 13 nmol/min per ml GFR added to renal venous blood and 140 \pm 13 nmol/min per ml GFR added to the urine. Therefore, the FE_{NH₃} was 50 \pm 5%. PGE₂ and PGF_{2 α} excretion in normal rats were 87 \pm 7 pg/min and 191 \pm 19 pg/min, respectively. Extrapolation of these data to 24 h yields values comparable to those reported by others for rats undergoing balance studies (19).

Low-dose meclofenamate (1 mg/kg). Administration of low-dose meclofenamate had no hemodynamic effect nor were urinary or arterial acid-base parameters significantly altered. Arterial and renal venous ammonia concentrations were unchanged, while the renal venous, arterial ammonia difference was increased when compared with PG-intact rats. Total ammonia production significantly increased from 281 \pm 19 to 591 \pm 46 nmol/min per ml GFR (*P* < 0.01), *pari passu* with the diminished PG synthesis (Fig. 2). While ammonia addition to the renal vein and urine both increased, the urinary component rose proportionately more, with FE_{NH₃} increasing from 50 \pm 5 in controls to 61 \pm 5% in PG-depleted rats (*P* < 0.05). The enhancement of the FE_{NH₃} was independent of changes

Table I. Clearance Studies: Acid-Base Parameters, In Vivo Ammonia and PG Production in Rats with Normal Acid-Base Status

Physiologic and chemical parameters	PG-intact (11)*	Meclofenamate-induced PG depletion	
		Meclo 1/mg/kg (12)	Meclo 5 mg/kg (9)
Clearance and acid-base parameters			
C _{IN} (ml/min per 0.1 kg)	1.05±0.04	0.98±0.05	0.89±0.08§
RBF (ml/min per 0.1 kg)	4.81±0.25	4.56±0.29	3.78±0.95
Vol (μl/min)	66±10	73±10	35±5
Urine pH	6.35±0.10	6.48±0.21	6.49±0.13
Arterial pH	7.39±0.01	7.40±0.01	7.40±0.01
HCO ₃ (mM)	22.9±0.5	22.8±0.54	22.1±0.5
PCO ₂ (torr)	38.4±1.3	38.7±1.1	36.3±1.4
Ammonia and PG production			
Arterial [NH ₃] (nmol/ml)	69±5	61±4	68±11
Renal venous [NH ₃] (nmol/ml)	100±6	110±7	135±15§
RV - A[NH ₃] (nmol/ml)	31±2	49±7§	65±13
NH ₃ added to RV‡	140±13	227±26§	283±58
Urinary excretion NH ₃ ‡	140±13	354±20	483±58
Total NH ₃ production‡	281±19	591±46	766±87
FE _{NH₃} (%)	50±5	61±5§	63±8§
PG excretion (pg/min)			
PGF _{2α}	191±19	84±4	UD
PGE ₂	87±7	34±5	UD

All data are expressed as means \pm SE. RV - A[NH₃], renal venous, arterial difference in ammonia concentration; C_{IN}, clearance of inulin; Meclo, meclofenamate, RV, renal vein; UD, undetectable; Vol, urine volume.

* No. of rats in each group.

‡ Values expressed in nanomoles per minute per milliliter GFR (means \pm SE).

|| *P* < 0.01; § *P* < 0.05. *P*-values refer to differences between PG-intact and PG-depleted rats.

in urinary pH or urine flow rates. PGE₂ and PGF_{2 α} synthesis were each decreased by 44% (*P* < 0.01), to 34 \pm 5 and 84 \pm 4 pg/min, respectively.

High-dose meclofenamate (5 mg/kg). The GFR and RBF were reduced by high-dose meclofenamate to ~80% of values found in normal controls and rats receiving low-dose (1 mg/kg) meclofenamate. There were no changes in mean arterial pressure, or in urinary or arterial acid-base parameters (Table I). High-dose meclofenamate increased the rate of addition of

Table II. Clearance Studies: Acid-Base Parameters, Ammonia, and PG Production in Rats with Metabolic Acidosis and Alkalosis

Physiologic and chemical parameters	AMA				Acute metabolic alkalosis	
	Mild acidosis		Severe acidosis			
	PG-intact (7)*	PG-deplete (11)	PG-intact (13)	PG-deplete (8)	PG-intact (6)	PG-deplete (7)
Clearance and acid-base parameters						
C _{IN} (ml/min per 0.1 kg)	0.79±0.08	0.91±0.07	0.89±0.07	0.85±0.08	0.90±0.02	0.93±0.06
RBF (ml/min per 0.1 kg)	3.70±0.27	4.48±0.63	3.93±0.32	4.53±0.54	4.90±0.42	4.49±0.45
Vol (μl/min)	46±10	33±6§	49±9	23±2§	103±23	67±11§
Urine pH	6.02±0.13	6.28±0.16	5.77±0.08	6.11±0.05§	7.73±0.22	7.45±0.04
Arterial pH	7.29±0.01	7.32±0.01	7.23±0.02	7.23±0.01	7.53±0.01	7.50±0.01
HCO ₃ (mM)	18.0±0.4	19.8±0.9	14.2±0.8	13.9±0.8	31.8±0.8	32.4±0.4
PCO ₂ (torr)	38.2±1.1	38.0±1.6	34.7±1.5	34.2±2.1	40.4±1.3	39.5±1.1
Ammonia and PG production						
Arterial [NH ₃] (nmol/ml)	95±8	56±8§	101±12	91±17	38±12	63±8§
Renal venous [NH ₃] (nmol/ml)	165±14	126±7§	165±13	168±15	93±17	127±11§
RV – A[NH ₃] (nmol/ml)	70±9	70±14	54±11	77±12	55±6	64±8
NH ₃ added to RV‡	302±59	350±53	350±57	349±33	234±33	234±48
Urinary excretion NH ₃ ‡	315±35	503±50	483±60	503±67	96±7	183±48§
Total NH ₃ production‡	617±67	853±88§	833±102	852±74	330±29	417±38§
FE _{NH₃} (%)	51±3	59±4§	58±3	59±6	29±6	44±5
PG excretion (pg/min)						
PGF _{2α}	695±178	68±11	545±81	67±13	176±39	UD
PGE ₂	256±42	51±9	336±18	29±8	59±22	UD

Abbreviations and symbols as in Table I. PG depletion was induced with the intravenous infusion of 5 mg/kg of meclofenamate.

ammonia to the renal vein to values that significantly exceeded those in normal controls. While the rate of ammonia addition to the renal vein exceeded that found in rats receiving low-

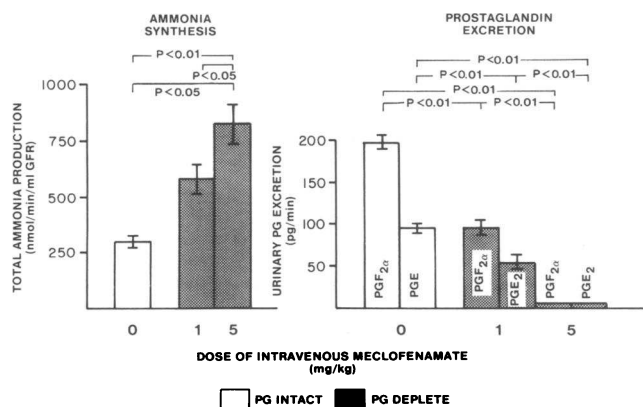


Figure 2. Response of renal ammoniagenesis to increasing doses of meclofenamate. Differences in ammonia production and PG excretion are indicated above the bars.

dose meclofenamate, the difference was not statistically significant (Table I). The urinary excretion of ammonia, however, was significantly greater in rats on high-dose drug than in those receiving low dose ($P < 0.05$). Thus, total ammonia synthesis was increased by high-dose meclofenamate (766 ± 87 nmol/min per ml GFR) to values in excess of those found in rats given low dose meclofenamate (591 ± 46 nmol/min per ml GFR; $P < 0.05$). The FE_{NH_3} was increased by meclofenamate but the increment was not dose dependent. Again, the meclofenamate-induced increase in the FE_{NH_3} was independent of any change in urinary pH or flow rate. The increased production of ammonia and decreased production of PGs that were elicited by increasing doses of meclofenamate is depicted in Fig. 2. The negative-inverse relationship between ammonia synthesis and PG production achieved high degrees of statistical significance: $y = -2.44x + 776$; $r = 0.78$ ($P < 0.01$) for PGF_{2α} and $y = -4.6x + 741$; $r = -0.65$ ($P < 0.01$) for PGE₂ (Fig. 3).

Indomethacin-treated normal rats. To reduce the possibility that the ammoniagenic response to meclofenamate was a nonspecific chemical effect unrelated to PG inhibition, indomethacin, a structurally dissimilar inhibitor, was studied. Five

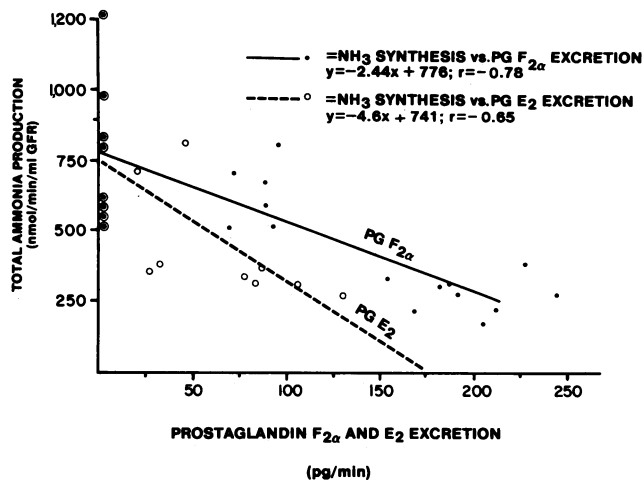


Figure 3. Relationship between total ammonia production and urinary PG excretion. (•), $\text{PGF}_{2\alpha}$; (○), PGE_2 ; (⊙), overlapping values of NH_3 production when both PGs were undetectable. The slopes are significantly different from each other ($P < 0.05$) and the correlation coefficients are significant ($P < 0.01$).

rats received 5 mg/kg of indomethacin i.v., 60 min before urine and blood collections were made. Indomethacin caused no significant change in mean arterial pressure, GFR (1.03 ± 0.05 ml/min per 0.1 kg), or RBF (5.08 ± 0.25 ml/min per 0.1 kg) as compared with high-dose meclofenamate-treated rats. Acid-base parameters and the reduction of urinary PGE_2 and $\text{PGF}_{2\alpha}$ excretion were equally affected by both inhibitors. Total ammonia production was increased (731 ± 50 nmoles/min per ml GFR) by indomethacin to levels equal to those found in high-dose meclofenamate-treated rats. Indomethacin, like meclofenamate, increased the FE_{NH_3} .

AMA

Mild AMA, PG intact (Table II; Fig. 4). Hydrochloric acid (2 mmol/kg) was infused over 40 min, which resulted in mild but significant decreases in arterial pH, bicarbonate, and urinary pH (Table II). Although mean arterial pressures were unchanged, both GFR and RBF decreased from control values (Table II). The arterial and renal venous ammonia concentration and the renal venous, arterial ammonia difference were increased to values greater than those of control, PG-intact, nonacidemic rats. Mild acidemia resulted in more than a twofold increase in total ammonia synthesis as compared with controls (617 ± 67 vs. 281 ± 19 nmoles/min per ml GFR; $P < 0.01$; Fig. 4). Mild AMA dramatically increased the excretion of PGE_2 from 87 ± 7 to 256 ± 42 pg/min ($P < 0.01$) and $\text{PGF}_{2\alpha}$ from 191 ± 19 to 695 ± 178 pg/min ($P < 0.01$) (Table II). The FE_{NH_3} during AMA was 51 ± 3 , a value no different from normal rats. One might have anticipated that the FE_{NH_3} would increase in mAMA. The urine flow rate dramatically fell and PG excretion significantly increased, however, and as will be

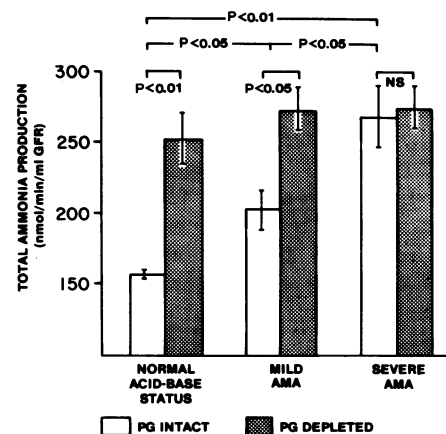


Figure 4. Ammonia production and PG excretion in normal rats and those with mAMA and sAMA. PG depletion was induced with 5 mg/kg of intravenous meclofenamate. Intergroup differences are indicated by the P -values above the bars. Corresponding PG excretion can be found in Table II.

discussed below these forces may well underlie the failure to demonstrate an increased FE_{NH_3} .

PGs appear to inhibit renal ammonia synthesis, since acute suppression of their production strikingly stimulates ammoniogenesis. Mild AMA simultaneously stimulated ammonia generation and the production of inhibitory PGs. The following experiments were therefore carried out to define whether a given degree of acidosis can provoke a greater degree of ammoniogenesis if PG synthesis is inhibited.

Meclofenamate plus mild AMA (Table II; Fig. 4). The administration of 5 mg/kg of meclofenamate 30 min before the induction of mAMA did not alter RBF, GFR, or the systemic or urinary acid-base response to HCl loading when compared with PG-intact rats receiving a similar load of acid (Table II; Fig. 4). Arterial (56 ± 8 nmol/ml) and renal venous (126 ± 7 nmol/ml) ammonia concentrations were less than those seen in PG-intact rats undergoing mAMA ($P < 0.05$), but the renal venous, arterial ammonia differences were the same in both groups. Unlike normal acid-base conditions where high-dose meclofenamate was able to erase PGs from the urine, this dose administered to rats undergoing mAMA could only lower urinary levels to 51 ± 9 pg/min and 68 ± 11 pg/min, for PGE_2 and $\text{PGF}_{2\alpha}$, respectively. Prevention of the acid-induced increment in PG synthesis further augmented the increase in ammoniogenesis elicited by mAMA. Total ammonia production in PG-intact rats with mAMA was 617 ± 67 nmol/min per ml GFR but was 853 ± 88 nmol/min per ml GFR ($P < 0.05$) in PG-deplete rats with mAMA (Fig. 4). This increased ammoniogenesis was associated with an increase in urinary ammonia excretion, while the ammonia added to the renal vein was no different than in PG-intact mAMA rats. In fact, the FE_{NH_3} significantly increased from 51 ± 3 to $59 \pm 4\%$ ($P < 0.05$) in PG-deplete mAMA rats. This

is similar to the alteration of FE_{NH_3} seen in PG-depleted normal rats.

sAMA (Table II; Fig. 4). During severe AMA, RBF and GFR slightly but significantly decreased as compared with control rats or rats with mAMA. Despite the increased severity of acidosis, urinary PG excretion did not increase beyond that seen in mAMA (Table II). Arterial and renal venous ammonia concentrations were not greater than values obtained in mAMA but were, of course, greater than those in normal rats. Total ammonia production increased to 833 ± 102 nmol/min per ml GFR, a value that was 30% greater than that seen in mAMA ($P < 0.05$). The increased ammonia synthesized by the kidney during sAMA exited primarily via the urine, and this was reflected by an increase in the FE_{NH_3} ($58 \pm 3\%$), which is significantly greater than PG-intact normal pH ($P < 0.05$) and PG-intact mAMA ($P < 0.05$).

Meclofenamate plus sAMA. The inhibition of PG synthesis before the administration of acid did not alter mean arterial pressure, RBF, GFR, systemic pH, arterial bicarbonate concentration, urinary pH, or urinary flow rates in comparison with PG-intact sAMA (Table II). PG excretion (PGE_2 , 29 ± 8 pg/min; $PGF_{2\alpha}$, 67 ± 13 pg/min) was significantly decreased but as in mAMA, measurable amounts were still present in the urine despite high-dose meclofenamate (Table II). In contrast to mAMA, inhibition of PG synthesis did not further augment the ammoniagenic response to sAMA. The rates of addition of ammonia to the renal vein and to the urine were equal in PG-intact and -depleted rats with sAMA.

When total ammonia production was plotted against $PGF_{2\alpha}$ excretion in normal rats and in rats with mild and severe metabolic acidoses, three distinct slopes were noted ($P < .01$ for normal vs. mAMA; $P < .05$ for mAMA vs. sAMA; Fig.

5). Corresponding correlation coefficients were significant for normal ($P < 0.01$) and mAMA ($P < 0.05$). Clearly, these data suggest that in normal rats and those with mAMA increasing renal net synthesis of $PGF_{2\alpha}$ reduces ammonia production. Severe AMA is not influenced by PG synthesis. The statistically significantly different slopes (Fig. 5) indicate that at any given level of $PGF_{2\alpha}$ synthesis ammoniogenesis increases with increasing degrees of acidosis.

Acute metabolic alkalosis. Alkalosis lowered the GFR to 0.90 ± 0.02 ml/min per 0.1 kg ($P < .05$) but RBF remained unchanged when compared with rats in normal acid-base balance. Urine flow rates were increased (103 ± 23 μ l/min) as was systemic pH, arterial bicarbonate concentration, and urine pH. Arterial ammonia concentration was lower and the renal venous, arterial difference was increased in comparison with values obtained from normal controls. Total ammonia synthesis was not decreased by acute alkalosis although the urinary excretion was diminished (96 ± 7 vs. 140 ± 13 nmol/min per ml GFR; $P < 0.05$). It follows that the rate of ammonia addition to renal venous blood increased (Table II). The FE_{NH_3} was $29 \pm 6\%$, which was significantly lower than normal rats ($50 \pm 5\%$; $P < 0.01$). Excretion of $PGF_{2\alpha}$ (176 ± 39 pg/min) and PGE_2 (59 ± 22 pg/min) were unchanged by alkalosis.

PG-deplete rats with AMA. Inhibition of PG synthesis did not significantly alter the GFR or RBF but did reduce urine flow rates (67 ± 11 μ l/min) in acutely alkalotic rats (Table II). Arterial pH, bicarbonate concentration, and urinary pH were not different from values obtained in alkalotic, PG-intact rats. Meclofenamate administration reduced urinary PG (PGE_2 and $PGF_{2\alpha}$) excretion in alkalotic rats to unmeasurable values (Table II). Arterial ammonia concentration was increased to normal (63 ± 8 nmol/ml), while the renal venous, arterial

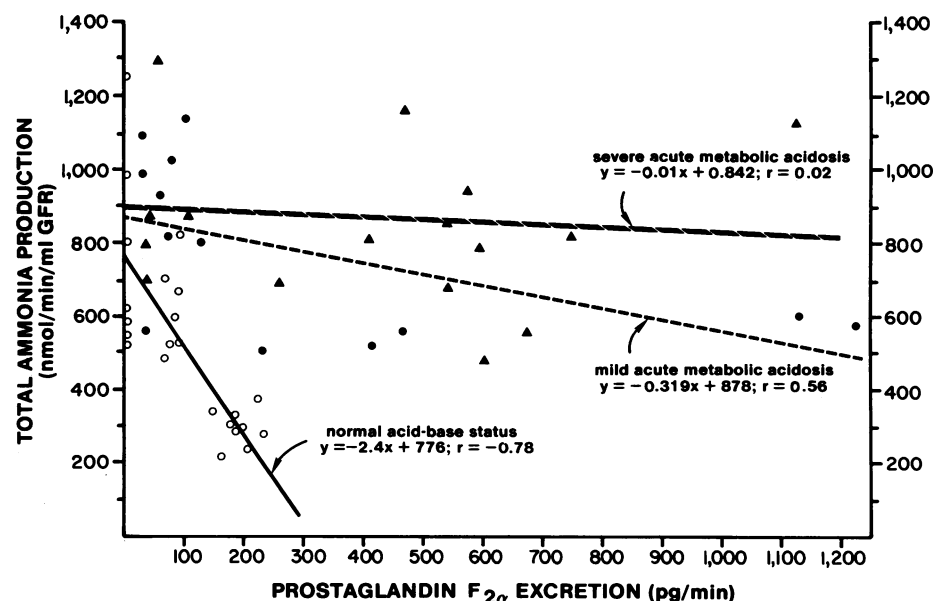


Figure 5. Relationship between total ammonia production and urinary $PGF_{2\alpha}$ excretion in rats under normal acid-base conditions and in those undergoing mAMA and sAMA. (○) and solid line refer to normal acid-base status; (●) and thin broken line, to mild AMA; and (Δ) and heavy broken line, to severe AMA.

Table III. Ammonia and PG Production by Incubated Rat Renal Cortical Slices

Groups	Tissue weight	Total NH ₃ production	PGF _{2α} production
	mg dry wt	μmol/g dry weight/h	ng/mg dry wt/h
PG intact (15)*	18.2±0.2	240±22	142±22
PG deplete (18)	16.5±0.1	309±15	66±12
PG excess (9)	18.5±0.2	205±12	335±58

In vitro PG synthesis was inhibited by adding meclofenamate (PG deplete) or stimulated by adding calcium ionophore (PG excess), or vehicle (PG intact).

* The number in parenthesis refers to the number of experiments.

^{||} $P < 0.5$, paired t test.

P -values refer to the comparison of PGs intact slices with PGs excess or PGs deplete slices.

ammonia differences remained unchanged when PG synthesis was inhibited in alkalotic rats. Total ammonia production increased significantly from 330 ± 29 nmol/min per ml GFR in PG-intact alkalotic rats to 417 ± 38 nmol/min per ml GFR in PG-deplete, alkalotic rats ($P < 0.05$). The FE_{NH_3} returned to normal after PG synthesis inhibition ($44 \pm 5\%$). The increment in total ammonia synthesis effected by PG inhibition in alkalotic rats was substantially less than the increment produced in normal, nonalkalotic rats by meclofenamate (87 nmol/min per ml GFR vs. 485 nmol/min per ml GFR; $P < 0.01$).

In vitro studies. Pooled renal cortical slices from normal rats were randomly distributed into three media: standard KHB (PG intact), KHB plus meclofenamate (PG depleted), and KHB plus calcium ionophore, to stimulate PG synthesis (PG excess). The in vitro reduction of PG synthesis was associated with increased ammonia production (Table III), albeit not to unmeasurable levels. Indomethacin produced similar results; PGF_{2α} synthesis fell from 178 ± 28 to 81 ± 19 ng/g dry wt per h while NH₃ synthesis increased from 221 ± 30 to 298 ± 18 μmol/g dry wt per h; $P < 0.05$. Calcium ionophore moderately but significantly increased PG production and simultaneously inhibited ammonia synthesis. On acidification of the incubation medium (pH 7.0), no significant changes in ammonia synthesis (314 ± 17 vs. 271 ± 37 μmol/g dry wt per h) were noted, however, PGF_{2α} production increased by 32% (264 ± 35 vs. 356 ± 65 ng/d dry wt per h; $P < 0.05$).

Discussion

Although our understanding of how renal ammoniogenesis is controlled has greatly increased over the past 20 years, much remains to be explained. Systemic pH, serum potassium concentration, adrenal steroids, and RBF have been extensively studied and shown to play key regulatory roles (4). The means by which changes in acid-base or potassium balance are translated into salutary alterations in renal ammonia synthesis

remain largely unknown. Indeed, it is uncertain whether these effectors act directly on renal metabolic pathways or if a second messenger such as PGs, mediates the observed changes.

PGs are important regulators of RBF, GFR, renin release, and renal salt and water handling (1). The interaction between PGs and renal ammoniogenesis has not, however, been systematically studied. Herein, we report for the first time that PGs are potent inhibitors of renal ammonia synthesis.

We have demonstrated that administration of high doses of PG-synthesis inhibitors to rats with normal blood pH simultaneously eradicated PGF_{2α} and PGE₂ from the urine and effected a threefold increase in total ammonia production. The causal relationship between reduced PG synthesis and increased ammonia production is strengthened by the fact that two chemically dissimilar inhibitors, meclofenamate and indomethacin, equally stimulated ammoniogenesis. Additionally, the dose response of ammonia produced to meclofenamate administered revealed a very strong correlation between the fall in PG excretion and the rise in ammonia generation (Fig. 3). Finally, not only did the addition of PG inhibitors to incubated renal cortical slices fully reproduce the stimulation of ammoniogenesis found in vivo, but in vitro stimulation of PG synthesis inhibited basal ammonia production (Table III). This inverse relationship therefore suggests that production of ammonia may be partially but constantly suppressed by the local synthesis of PGs.

Certain insights into the mechanisms by which PGs may influence ammonia synthesis can be gleaned from our data. That in vitro modification of PG synthesis resulted in qualitatively similar changes in ammoniogenesis as seen in vivo, suggests that the PG effect is likely to be independent of hemodynamic changes, GFR, or ambient concentration of glutamine, potassium, calcium, protons, or extrarenal hormones. The independence of the PG effects from renal hemodynamic changes is strengthened by the fact that inhibitors of PG synthesis stimulate ammonia production but tend to reduce RBF and GFR (20). The latter effect ought to diminish, not enhance, ammoniogenesis (21). While the PG-ammonia effect clearly takes place in the cortex, we have yet to identify the exact anatomic site of this interaction.

Calcium may have a more influential role in mediating the interplay between PGs and ammonia synthesis. Calcium ionophore A23187 facilitates cellular uptake of the divalent cation and simultaneously stimulates PG synthesis (22). Hypercalcemia has been variously reported to increase (23), decrease (24), or leave renal ammoniogenesis unchanged (25). However, renal PG production has been consistently shown to vary inversely with the cellular uptake of calcium; nifedipine, the calcium channel blocker, inhibits both processes and calcium ionophore A23187, enhances them (22). Thus, forces regulating renal epithelial cell calcium concentration could influence ammoniogenesis via the intermediation of altered PG synthesis. The role, if any, of calcium-PGs in translating alterations in the concentration of serum electrolytes or hormones into changes in ammoniogenesis remains to be explored.

Renal ammonia synthesis is critically dependent upon the continued availability of glutamine, the kidney's preferred source of ammonia nitrogen (26). The accessibility of this amino acid is dependent upon its extrarenal production and utilization, delivery to the kidney, and renal epithelial cell uptake and intracellular distribution. It is unlikely that the demonstrated effects of PGs on ammonia synthesis were influenced by extrarenal alterations in glutamine metabolism. Serum glutamine concentration was unchanged after acute administration of PG synthesis inhibitors ($446 \pm 10 \mu\text{M}$ in normal rats vs. $418 \pm 65 \mu\text{M}$ in rats after 1 mg/kg meclofenamate; unpublished observation) and RBF, if changed at all, fell slightly. Thus, the product of RBF and serum glutamine concentration did not increase, which eliminated enhanced substrate delivery as an explanation of how removal of PGs stimulated renal ammoniogenesis. While PGs may influence the cellular uptake of glutamine, neither our studies nor any published work has addressed this potentially important issue.

The cellular effects of PGs are thought to be negotiated primarily at the cell membrane and little is known of any direct intracellular metabolic effects (1). The PGs could inhibit ammoniogenesis by impairing cytosolic and/or mitochondrial reactions involved in stripping nitrogen from glutamine and/or those concerned with disposing of its carbon skeleton. These possibilities are currently being explored.

In addition to their influence on total renal production, the PGs also modified the distribution of ammonia outflow from the kidney. Inhibition of PG production in normal rats increased ammoniogenesis from $281 \pm 19 \text{ nmol/min per ml GFR}$ to $766 \pm 87 \text{ nmol/min per ml GFR}$ and concomitantly increased the fraction of exiting ammonia that appeared in the urine (i.e., the FE_{NH_3}) from 50 ± 5 to $63 \pm 8\%$ ($P < 0.05$). This augmented urinary capture of ammonia occurred in the absence of such factors as aciduria and polyuria, which are known to enhance the FE_{NH_3} . Recent studies describing the medullary trapping of ammonia offer a reasonable explanation for our findings (27, 28).

It has been shown that progressive water loss from the glomerular filtrate, as it descends through the increasingly more concentrated medulla, renders remaining fluid more alkaline by increasing the bicarbonate concentration in the descending limb (27, 28). The alkalization converts luminal NH_4^+ to NH_3 , which in turn passes through the medullary interstitium and is trapped in the relatively more acidic environment of the medullary collecting system (27, 28). This proximal tubule-to-loop of Henle-to-collecting duct pathway short-circuits return of NH_3 to the cortex and ensures its efficient excretion.

It follows that factors that enhance the concentration of solute in the medullary interstitium ought to enhance the egress of water from the descending limb, thereby augmenting ammonia trapping. The PGs may well increase medullary blood flow (29, 30) and inhibit sodium reabsorption by the thick ascending limb (31). It is reasonable to expect that inhibition of PG synthesis would allow more sodium chloride

to enter the interstitium and the associated reduction in medullary blood flow would prevent washout of interstitial solute. This scenario could easily explain why indomethacin and meclofenamate both enhance the FE_{NH_3} . Further studies are needed to explore this hypothesis.

While metabolic acidosis and alkalosis are well-known modifiers of renal ammoniogenesis, it was uncertain whether associated changes in PG synthesis influenced this interplay. Indeed, a number of previously published studies suggested that acid-base changes did modify PGs synthesis (5–10).

Studies by Gulyassay and Edelman (8) as well as others (5–7) showed that acidification of the serosal media bathing toad bladders inhibited the hydro-osmotic effect of ADH (5–7). Most recently, Forrest et al. (9) confirmed these results and further noted that serosal acidification increased PGE_2 production. The addition of meclofenamate or indomethacin suppressed PG production by the acidified bladder and restored the hydro-osmotic effect of ADH. In concert with these *in vitro* studies, Beck and Kim (10) showed that in comparison with normal rats, those made chronically acidotic could not appropriately elevate their urinary osmolality in response to administered vasopressin. These observations strongly suggest that modest degrees of metabolic acidosis stimulate PG synthesis and the latter evoke physiologic changes.

We demonstrated that 40 min after the induction of mAMA, total renal ammonia production by anesthetized rats more than doubled, while excretion of PGE_2 and $\text{PGF}_{2\alpha}$ increased by 2.9–3.6-fold (Table II). Since our studies of normal rats indicated that PGs inhibited ammoniogenesis, we reasoned that acidosis-stimulated ammonia synthesis must reflect the algebraic sum of direct acid enhancement and the indirect inhibition of augmented PG synthesis. Reduction of acid-stimulated PG production with various doses of meclofenamate caused further enhancement of ammonia production (Fig. 5), which corroborated the above postulate. Thus, over a rather narrow range of metabolic acidosis (serum HCO_3^- : $18.0 \pm 0.4 \text{ mM}$, arterial pH: 7.29 ± 0.01), inhibitor-induced alterations in PG excretion changed total ammonia production by $\sim 50\%$ (Fig. 4). Failure of more severe degrees of hypobicarbonatemia to further enhance PG synthesis suggests that maximal stimulation was achieved when the serum bicarbonate concentration reached 18 mM. Furthermore, it would appear that the direct stimulatory effect of the more severe degrees of acidosis are powerful enough to neutralize the inhibitory action of stimulated PG synthesis. This situation seems analogous to the diminishing effect of inhibited PG synthesis in augmenting the hydro-osmotic effect of increasing doses of ADH (32). This hormone simultaneously enhances water reabsorption from the collecting duct while stimulating PG synthesis, which, in turn reduces the magnitude of ADH's effect. As in severe acidosis, the direct action of high doses of the hormone is no longer enhanced by PG synthesis inhibition (32, 33).

As in normal rats, the PGs strongly influenced both total ammonia production and the FE_{NH_3} in mAMA. Meclofenamate simultaneously stimulated total synthesis and increased the

FE_{NH₃} by 8% (Table II). As with normal rats, the enhanced excretion occurred in the absence of a fall in urinary pH, and urinary volume decreased, which ought to diminish, not increase, the FE_{NH₃}. We would again speculate that the diminution in medullary blood flow and enhanced solute entry into the medulla consequent to inhibition of PG synthesis, enhances the medullary solute concentration and ammonia trapping.

The mechanism by which acidosis stimulates renal PGs synthesis was not addressed by our studies. Future studies will evaluate the influence of acidosis on arachadonic acid mobilization and on the conversion of the fatty acid into the various PGs.

After 40 min of sustained acute metabolic alkalosis total ammonia synthesis was not changed but the alkaline urinary pH strikingly reduced the FE_{NH₃} from the normal 50±5 to 29±6% (Table II). Presumably, a longer duration of alkalosis is required to inhibit ammonia synthesis.

AMA had no effect on PG excretion. However, inhibition of PG synthesis, was associated with a small but significant increase in total ammonia production, which indicates that PGs also modulate ammoniogenesis during mild alkalemia. It should be noted that virtually the entire increment in ammonia production caused by meclofenamate was excreted in the urine. The increase in the FE_{NH₃} from 29±6 to 44±5% enabled the alkalotic kidney to produce and excrete an additional 88 nmol/min per ml GFR. Inhibition of PG synthesis in normal rats increased ammoniogenesis by 485 nmol/min per ml GFR and in mildly acidotic rats by 236 nmol/min per ml GFR but similar inhibition of PG synthesis only increased ammonia production by 88 nmol/min per ml GFR in alkalotic rats. Thus, alkalosis appears to curb meclofenamate stimulation of ammoniogenesis.

Our concept of the interplay between PGs and ammonia synthesis is summarized as follows. The PGs appear to act as negative-feedback inhibitors of ammonia synthesis, functioning in a manner similar to that observed in other biological systems. A number of stimuli such as vasopressin, angiotensin II, and acidosis exert primary stimulatory effects on the kidney, which causes reabsorption of water, vasoconstriction or enhances ammoniogenesis; these stimuli increase PG synthesis, which in turn inhibit the direct effect of the stimuli. Thus, augmented release of PGs inhibits the hydro-osmotic effect of ADH, causes vasodilatation by countering angiotensin II's effect, and now we have demonstrated that PGs inhibit ammonia synthesis. If the PGs effect is eradicated, the primary effect on the kidney is enhanced. However, pharmacologic doses of the primary effectors, e.g., ADH (32, 33) or angiotensin II (34, 35) can override the inhibitory effect of PGs as we have noted in severe metabolic acidosis. This regulatory function of PGs on ammonia synthesis appears to act most efficiently during normal acid-base conditions and with mild changes of pH.

We conclude that PGs inhibit ammonia synthesis. It remains to be shown whether stimuli other than mAMA influence ammoniogenesis by altering renal PG synthesis.

Acknowledgments

We appreciate the excellent secretarial assistance of Ms. Elsie M. Williams.

This work was supported by a grant from the Southeastern Chapter of the National Kidney Foundation and a Clinical Investigation Award from the National Institutes of Health (1 K08 AM01211-01).

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