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PERSPECTIVES IN BASIC SCIENCE

Regulatory and functional interaction of vasoactive factors in the kidney and extracellular pH

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Regulatory and functional interaction of vasoactive factors in the kidney and extracellular pH. A growing body of evidence suggests that vasoactive factors produced in the kidney such as nitric oxide, endothelins, angiotensin, and prostaglandins participate actively in the regulation of acid-base homeostasis under physiologic conditions. In addition, recent reports indicate that alterations in the systemic acid-base status may also influence the generation of vasoactive cytokines in the kidney, which in turn may mediate the renal effector processes that tend to restore normality under such conditions. Metabolic acidosis, which so frequently accompanies many forms of chronic renal failure (CRF), may contribute to down-regulation of intrarenal nitric oxide production that characterizes CRF. Reduced extracellular pH inhibits inducible nitric oxide production in mesangial cells by altering the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) oxidation, an important post-translational mechanism in the inducible nitric oxide synthase (iNOS) activation. The underlying defects resulting in the uncoupling of NADPH oxidation in acidemic microenvironment are discussed. Acidosis stimulates renal production of endothelins, which mediate proximal tubular acidification by enhancing sodium-hydrogen exchanger-3 (NHE-3) activity. Renal endothelins mediate enhanced urinary acid excretion following dietary acid ingestion, an effect that is effectively blocked by endothelin receptor blockers. Reduced extracellular pH stimulates endothelin secretion from renal microvascular endothelial cells, which may promote enhanced acid excretion from the distal tubule under conditions of acidosis. These phenomena as well as the role of angiotensin and renal prostaglandins in mediating renal acidification in normal and acidotic conditions are discussed in this review, which describe the regulatory interaction between extracellular pH and renal vasoactive factors.

Recent literature has established the significance of vasoactive factors produced in the kidney in regulation of normal renal function as well as in the development and progression of renal disease through hemodynamic and nonhemodynamic effects. Such factors include nitric oxide, endothelins (ETs), prostaglandins, and angiotensin. Several experimental and clinical studies have supported the concept that activation of renin-angiotensin axis and down-regulation of L-arginine-nitric oxide pathway accelerate the renal failure caused by different etiologies, specifically diabetes [1, 2]. Acidosis, which is an important and integral feature of chronic renal failure (CRF), has been shown to influence the renal production of ETs and nitric oxide, an effect that can potentially contribute to the course of renal failure. In addition, alterations in the production of vasoactive factors in the kidney may constitute important effector mechanisms, which mediate renal responses to the systemic acid-base disturbances. Moreover, in systemic conditions associated with acidosis such as septicemia and diabetic ketoacidosis, vasoactive factors mediate many clinical consequences of the primary disorder. This review attempts to provide an overview of the current understanding of the physiologic role of vasoactive factors produced by the kidney in systemic pH regulation and alterations in their production in response to systemic acid-base disturbances.

Key words: nitric oxide, endothelin, angiotensin, prostaglandins, extracellular pH.

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NITRIC OXIDE

Immediately following the identification of endothelial-derived relaxing factor (EDRF) as nitric oxide, the scientific literature was flooded with reports supporting the role of nitric oxide as a paracrine mediator of a variety of physiologic and pathologic effects in various organs systems. Specifically in the kidney, studies indicated that nitric oxide plays an important role not only in the control of renal hemodynamics, tubuloglomerular feedback, and glomerular filtration, but also in the regulation of tubular transport processes in various segments. Three isoforms of the enzyme, nitric oxide synthase (NOS) mediate the conversion of L-arginine to L-citrulline, which is the sole pathway for the generation of endogenous nitric oxide. All the three isoforms [neuronal NOS (nNOS or NOS I), inducible NOS (iNOS or NOS II) and endothelial NOS

(eNOS or NOS III)] are expressed differentially in various segments of nephron. nNOS and eNOS are traditionally considered as constitutively expressed and with limited tissue distribution, exhibiting intracellular Ca^{++}/calmodulin dependency. On the other hand, iNOS is an inducible enzyme, expressed in all nucleated cells and generate large bursts of nitric oxide in response to immunologic stimuli. iNOS is generally considered Ca^{++}-independent although the presence of Ca^{++} enhances its activity. In view of the high diffusibility, nitric oxide generated in renal microvasculature or tubular cells can affect the function in adjacent parts of the nephron, and thus function as a paracrine mediator.

Many experimental models of CRF and even clinical evidence indicate that intrarenal nitric oxide production is decreased in many forms of CRF [3] and may contribute to the progression of renal failure by accelerating glomerular and interstitial sclerosis [4]. Since metabolic acidosis is a major consequence of renal failure, it is important to understand the effects of acid-base changes on the nitric oxide production in the kidney. Although the regulation of nitric oxide synthesis in various organs and cell systems has been extensively studied, the literature on the effects of pH on nitric oxide synthesis is limited. The following paragraphs summarize the available literature on the role of nitric oxide in the renal acidification mechanisms under physiologic and pathophysiologic conditions and the effects of extracellular pH (pH_e) on nitric oxide production with special reference to intrarenal nitric oxide generation.

ROLE OF NITRIC OXIDE IN RENAL ACIDIFICATION

Recently published data supports the role of nitric oxide as an important regulator of acidification in the various segments of renal tubule. The proximal tubule, under normal conditions, is responsible for reabsorbing 70% to 90% of the filtered bicarbonate and 50% of the filtered chloride. Bicarbonate reabsorption is mediated by H^+ secretion across the apical membrane of the proximal tubule and occurs by two different mechanisms. The major fraction (60% to 70%), of H^+ secretion occurs by a Na^+-dependent, amiloride-inhibitable process mediated by an apical Na^+/H^+ antiporter or exchanger (NHE). The remaining 30% to 40% of the H^+ secretion occurs by a Na^+-independent amiloride-insensitive process, mediated by adenosine triphosphate (ATP)-driven H^+ transport. Bicarbonate reabsorption across the basolateral membrane is facilitated predominantly by an electrogenic $Na^+ - 3HCO_3^-$ cotransporter.

Murer, Hopfer, and Kinne [5] were the first to demonstrate that H^+ secretion may be directly linked to luminal Na^+, implicating Na^+/H^+ exchange. The first Na^+/H^+ antiporter was cloned by Sardet, Franchi, and Pouyssegur [6], which was subsequently named NHE-1. Since then, six additional mammalian isoforms of NHE have been cloned (NHE 2 to 7). Among the seven isoforms, only NHEs 1 to 4 are expressed in the kidney. NHE-1 and NHE-4 are expressed exclusively on the basolateral membrane. Practically all of the apical Na^+-H^+ exchange activity of the proximal tubule is accounted by NHE-3 isoform, as confirmed by molecular, immunocytochemical, and inhibitor sensitive studies [7].

The effects of nitric oxide on proximal tubular acidification mechanisms are controversial. Studies by Wang [8] showed similar increases in the HCO_3 transport with a nitric oxide donor, *S*-nitroso *N*-acetyl-*D,L,* Penicillamine (SNAP), as well as 8-bromocriptine guanosine monophosphate (BrcGMP), which mediated the effects of nitric oxide. These studies however showed that nitric oxide stimulated proximal tubular Na^+ and HCO_3 transport through cyclic GMP (cGMP)-linked pathway by augmenting NHE activity in the proximal tubule. The same investigators [9] later demonstrated that in nNOS knockout mice models the bicarbonate flux ($JHCO_3$) was significantly reduced compared to the wild-type and these animals developed metabolic acidosis. Similar observations were made in the iNOS knockout mice [10]. All of these studies indicated that endogenous nitric oxide from iNOS and nNOS promoted HCO_3 transport and H^+ secretion, while eNOS had no demonstrable effect on modulation of proximal tubular sodium and bicarbonate transport. Amorena and Castro [11] first indicated that nitric oxide from endothelial cells might be involved in the regulation of acidification mechanisms in the proximal tubule. They showed in co-culture studies of proximal tubular and endothelial cells, that L-arginine analog N-nitro-L-arginine methyl ester (L-NAME) blocked and cGMP enhanced the rate of H^+ flux (JH) in proximal tubules driven by carbachol. They concluded that the peritubular endothelium exerts control on the proximal tubular acidification by stimulating Na^+/H^+ exchanger activity in proximal tubule and that this effect was mediated by endothelial nitric oxide. But thus far there is no evidence that eNOS independently affects the acidification mechanisms in proximal tubule.

On the contrary, other studies have shown that nitric oxide inhibited fluid reabsorption and acidification in the proximal tubule. Using split-drop micropuncture technique, Eitle et al [12] reported that sodium nitroprusside (SNP), a nitric oxide donor, inhibited fluid reabsorption in proximal tubule. Furthermore, a few other studies demonstrated that nitric oxide inhibited Na^+/H^+ exchange in proximal tubule [13]. In both freshly isolated proximal tubular segments and in primary cultures of proximal tubular cells [14], SNP caused significant inhibition of apical Na^+/H^+ exchanger, an action mediated at least partly by cGMP. These investigators explained the discrepancy between their findings and those in NOS knockout studies cited earlier [10] by the following arguments. First, in nNOS knockout models, lack of nNOS

expression in the gastrointestinal tract, for instance, results in severe intestinal malformations that could lead to alterations in electrolyte transport [15]. Furthermore, the in vitro transport characteristics in isolated proximal tubule preparation may be modified by denervation, since neuronal activity modulates nitric oxide production. Additionally, L-NAME may cause a paradoxic increase in nitric oxide production in renal cortex [16] which is in agreement with the observations of Wang, Inglis, and Kalb [9]. The latter study demonstrated that intravenous infusion of L-NAME in mice as well as in in situ microperfusion of proximal tubule resulted in natriuretic and diuretic effects. These data strongly suggest that the effects of L-NAME on tubular transport are not only difficult to interpret but also may represent effects that are independent of their effects on nitric oxide production. Furthermore, immunocytochemical and molecular biologic studies failed to demonstrate the expression of nNOS and iNOS in the proximal tubule.

In summary, the available evidence relating to effects of nitric oxide in proximal tubule supports both stimulation and inhibition of NHE activity. The discrepant observations may relate to differences in the nitric oxide concentrations, a stimulatory effect seen in lower concentrations (in micromolar range) and inhibitory effect in higher (millimolar) concentrations. Direct measurements of nitric oxide concentrations in the kidney tubules in vivo have yielded low concentrations (110 nmol/L) in both early and late proximal tubules [17]. These concentrations are similar to those used in vivo microperfusion studies [8] and NOS knockout studies [9, 10] cited earlier, where a stimulatory effect of nitric oxide on fluid reabsorption (*Jv*), sodium reabsorption and NHE activity was demonstrated. Thus, under physiologic conditions, nitric oxide stimulates proximal tubular sodium reabsorption and H^+ secretion through increased NHE activity. While nitric oxide derived from nNOS and iNOS seem to mediate such effects, there is no evidence that eNOS-derived nitric oxide plays any independent role in proximal tubular acidification and sodium reabsorption.

Studies by Ruiz et al [18] have shown that nitric oxide might modulate the activity of Na^+-$3HCO_3$ cotransporter in the rabbit proximal tubule. Carbachol, a cholinergic agent, increases nitric oxide production in proximal tubules. They showed that L-NAME, a NOS inhibitor, prevented the augmentation of the activity of Na^+-$3HCO_3^-$ cotransporter that is normally seen with carbachol. Nitric oxide thus plays a role in the renal acidification mechanisms in the proximal tubule by modulating the activity of Na^+-$3HCO_3^-$ cotransporter.

In conclusion, despite conflicting data, majority of evidence points out that nitric oxide stimulated Na^+ and HCO_3^- reabsorption by stimulating Na^+/H^+ exchanger and through augmenting basolateral electrogenic Na^+-$3HCO_3^-$ cotransporter. Thus, nitric oxide participates in the proximal tubular acidification mechanisms by at least two distinct mechanisms. Currently, there are no data implicating nitric oxide modulation of HCO_3^- reabsorption through electrogenic H^+ secretion.

In the medullary thick ascending limb of loop of Henle (mTALH), nitric oxide inhibited net Cl^- and HCO_3^- absorption by modulating the activity of the Na^+-K^+-$2Cl^-$ cotransporter and Na^+/H^+ exchanger [19]. Ortiz, Hong, and Garvin demonstrated that nitric oxide inhibited Na^+/H^+ exchanger activity at both apical and basolateral sides of the mTALH. By using a nitric oxide donor spermine NONOate (Cayma Chemical Ann Arbor, MI, USA), these investigators showed that nitric oxide decreased the intracellular pH. Furthermore, they measured the recovery from acid loading by measuring *J*H and found that spermine NONOate decreased *J*H in mTALH. The relative contributions of apical and basolateral Na^+/H^+ exchanger activity were determined by examining the effects of spermine NONOate on *J*H in mTALH in the presence of dimethyl amiloride in the bath or lumen. The data confirmed that nitric oxide inhibited both apical and basolateral Na^+/H^+ exchanger activity in mTALH. Consequently, nitric oxide may play an important role in bicarbonate reabsorption and thereby affecting renal tubular acidification in mTALH [20]. At the present time, there are no reports of nitric oxide modulating H^+ secretion in the distal tubule. In collecting duct, nitric oxide inhibits H^+ ATPase activity in the intercalated cells of the collecting duct [21].

Effects of pH_e on the nitric oxide production in the kidney

Clinical conditions in which the systemic and tissue acid-base status is altered impact significantly on cellular function. These functional changes may be mediated at least in part by alterations in local nitric oxide synthesis. The resultant changes in nitric oxide could be due to changes in expression or activity of NOS or due to decreased nitric oxide metabolites due to formation of reactive nitrogen species such as peroxynitrite. For example, acidosis stimulated nitric oxide synthesis in rat lung as measured by exhaled nitric oxide concentration [22]. On the other hand, studies by Bellocq et al [23] demonstrated that macrophages exposed to acidic pH (7.0), simulating the microenvironment of inflammation, showed increased iNOS activity through activation of nuclear factor-kappaB (NF-κB). Similarly, Unno, Hodin, and Fink [24] demonstrated stimulation of NOS activity in the presence of acidemia when the investigators exposed intestinal smooth muscle cells to decrease pH_e. However, in these experiments, nitric oxide generation was decreased due to the formation of peroxynitrite and hydroxyl radical formation, while the expression of iNOS protein itself was not affected. Reduced pH_e (7.0) decreased nitric oxide generation in inflammatory cells despite an increase in iNOS activity [25].

Cellular uptake of calcium has also been implicated in mediating the pH effects on NOS activity. Activity of eNOS is inhibited at reduced pH_e while it is increased with elevated pH_e. For instance, intracellular acidosis (pH 6.7) inhibited [26] and alkalosis stimulated eNOS activity in pulmonary endothelial cells by stimulating the influx of extracellular calcium flux [27]. In the latter studies, blockade of Na^+/Ca_2^+ exchanger with 3′4′ dichlorobenzamil hydrochloride prevented the increase in eNOS activity, suggesting that Na^+/Ca_2^+ exchanger regulates eNOS activity in acid-base disorders. In vascular smooth muscle, the effects of acidosis depend on the net effect of the pH on nitric oxide synthesis and the direct vasodilatory effect on the smooth muscle cells [28]. The latter may depend on the effects of pH_e on intracellular calcium, probably mediated by Ca^{++}/H^+ exchanger. Furthermore, the pH effects on vascular tone have been clarified in the light of pH sensitive florescent studies. It is likely that while decreased intracellular pH accounts for the immediate responses to acidosis, it is reduced pH_e that modulates the vascular tone in the steady state of acidosis [29]. In summary, the effects of pH on nitric oxide synthesis are variable and dependent on the cell type and NOS isoform involved and may be mediated in certain conditions by transcellular calcium fluxes.

The effects of changes in pH_e on nitric oxide generation specifically in the kidney are not well understood. Studies in rat proximal tubules suggested that reduced pH_e prevented hypoxia mediated nitric oxide synthesis [30]. Compared to a pH of 7.4, nitric oxide synthesis in proximal tubules was reduced at a pH of 7.0 despite hypoxia. These studies indicated that acidosis protects the proximal tubule from the deleterious effects of increased nitric oxide production resulting from hypoxia.

I hypothesized that pH_e regulates the inducible nitric oxide synthesis in the kidney. Specifically, I examined nitric oxide synthesis in mesangial cells [31] since nitric oxide derived from mesangial cells has been shown to modulate mesangial cell proliferation and matrix production [32]. My studies indicated that in murine mesangial cells in culture reduced pH_e inhibited inducible nitric oxide synthesis (Fig. 1) while alkaline pH (pH_a) did not affect mesangial nitric oxide production. The nitric oxide inhibition in acidosis was associated with decreased activity of iNOS, while iNOS gene and protein expression was unchanged from the control conditions. Further examination of the mechanisms underlining iNOS inhibition in acidic medium indicated that posttranslational factors such as tetrahydrobiopterin (BH_4) or substrate (L-arginine) supplementation did not influence the nitric oxide inhibition, while the oxidation of NADPH (reduced form of nicotinamide adenine dinucleotide phosphate) was significantly increased. Evaluation of $NADP^+$/citrulline stoichiometry under these conditions indicated uncoupling of NADPH oxidation

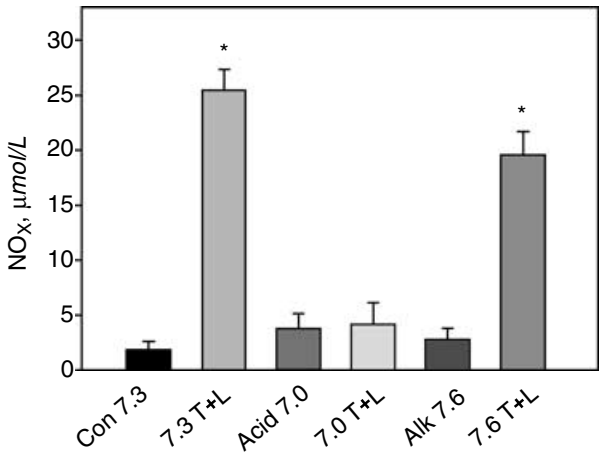

Fig. 1. Effects of extracellular pH (pH_e) on nitrate and nitrite (NO_x) accumulation in murine mesangial cells. Control pH (Con) is 7.3 while acidic and alkaline pH is 7.0 and 7.6, respectively. Abbreviations are: T, tumor necrosis factor-α (TNF-α), L, lipopolysaccharide (LPS). $^*P <$ 0.001 compared to control ($N = 12$ in each group). Reprinted from Prabhakar SS, *Kidney Int* 61:2015–2024, 2001. Permission requested.

(Fig. 2A). I concluded that acidosis inhibited iNOS activity by altering NADPH oxidation, an important posttranslational mechanism in iNOS activation. Currently, it is unclear whether increased oxidation of NADPH in reduced pH_e conditions, is due to activation of NADPH oxidase or due to the excess H^+ that may shift the NADPH $\rightarrow$ $NADP^+$ reaction to the right. My observations indicate that the latter may be the case (Fig. 2). Specifically, it is difficult to examine the role of NADPH oxidase activity since most inhibitors of NADPH also inhibit iNOS activity. My studies also demonstrated that under reduced pH_e conditions, saturating concentrations of NADPH normalized iNOS activity but not the NADPH-iNOS coupling. Thus, it is likely that increased rate of $NADP^+$ formation with resultant substrate (NADPH) depletion leads to reduced iNOS activation and uncoupling of NADPH oxidation.

Generation of nitric oxide from its precursor L-arginine involves cofactors such as BH_4, flavin mononucleotide (FMN), and flavin adenine dinucleotide (FAD), which shuttle electrons from the substrate NADPH at the enzyme site. This process occurs in two steps. The first step involves a two-electron oxidation supplied by one molecule of NADPH resulting in the formation of N-hydroxy L-arginine. In the second step, a second oxygen is supported by 0.5 molecule of NADPH leading to the formation of nitric oxide and citrulline. Thus, during the process of normally coupled iNOS activation, the NADP:citrulline stoichiometry is between 1.5 and 2.0. The iNOS activity is influenced by NADPH oxidation, which in turn is controlled by $NADP^+$/NADPH ratio in the cytosol. A potential explanation for increased generation of $NADP^+$ under reduced pH conditions may involve

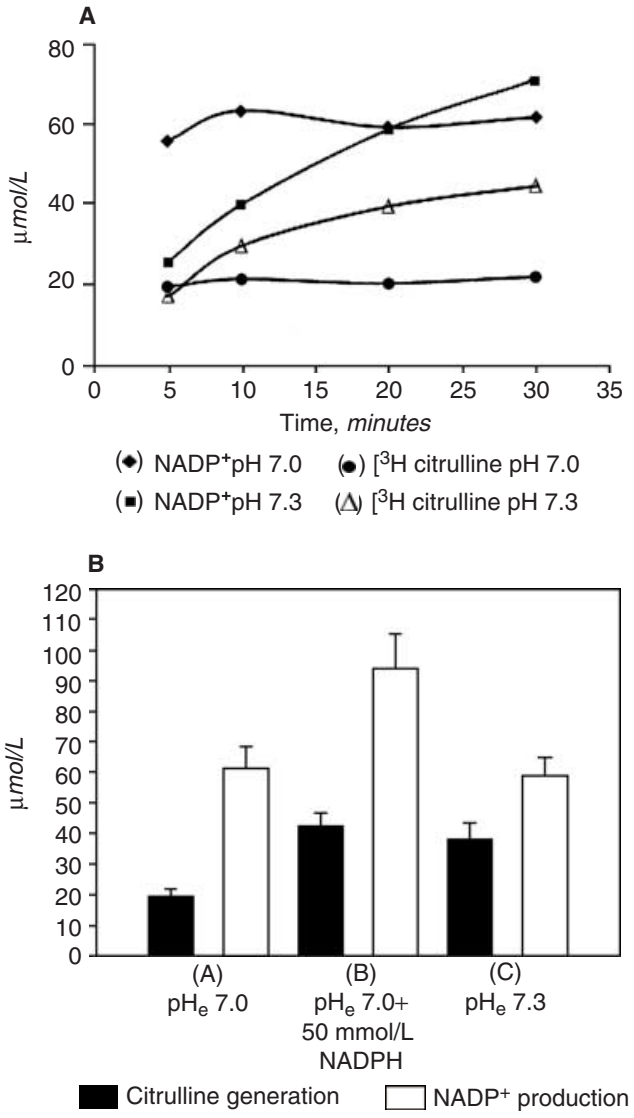

Fig. 2. Effects of pH_e on inducible nitric oxide synthase (iNOS)-reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) oxidation coupling. (*A*) Time course of iNOS activation and $NADP^+$ generation. Inhibition of iNOS activity seen with reduced exctracellular pH (pH_e) is associated with increased rate of NADPH oxidation with uncoupling of $NADP^+$/citrulline stoichiometry. The $NADP^+$ and citrulline generation are measured at several time intervals using the in vitro iNOS assay in reaction mixtures incubated at pH 7.0 and pH 7.3. As shown in the figure, excess H^+ in low pH_e conditions drive the reaction of NADPH → $NADP^+$ to the right. Symbols are: (◆) $NADP^+$ pH 7.0; (■) $NADP^+$ pH 7.3; (●) [^{3}H] citrulline pH 7.0; (△) [^{3}H] citrulline pH 7.3. The values shown represent the mean of four experiments in each group. (*B*) Citrulline-$NADP^+$ coupling. Effects of NADPH supplementation. Increased NADPH → $NADP^+$ conversion associated with reduced pH may lead to rapid depletion of NADPH accounting for decreased iNOS activity. To verify this hypothesis, $NADP^+$ (□) and citrulline generation (iNOS activity) (■) were measured in three sets of experiments that included (A) lysates from murine mesangial cells (MMC) stimulated at 7.0 pH, (B) lysates from MMC stimulated at pH 7.0 along with saturating (50 mmol/L) concentrations of NADPH, and (C) lysates from MMC stimulated at control (7.3) pH. NADPH concentration in groups (A) and (C) is 10 mmol/L ($N = 6$ in each group). $^*P < 0.01$ vs. corresponding result in group (A). (B) NADPH supplementation resulted in increased iNOS activity. However the uncoupling of NADPH → citrulline persisted. Reprinted from Prabhakar SS, *Kidney Int* 61:2015–2024, 2001. Permission requested.

a decreased $NADP^+$/NADPH ratio due to excess H^+, resulting in increased concentration of NADPH. Furthermore, the optimal pH for iNOS (7.0 to 8.0) is significantly higher than that for NADPH oxidation (5.5 to 6.5). Thus, uncoupling of iNOS activation and NADPH oxidation at reduced pH_e conditions is accounted for in part by the aforementioned differences in pK_a of NADPH oxidase and iNOS enzymes.

The pH-dependent uncoupling of iNOS could also be explained by destabilization of the iNOS homodimers under reduced pH_e conditions. Monomers of iNOS oxidize NADPH, but unlike dimers, monomers cannot synthesize nitric oxide. Recent literature suggests that nNOS activity is inhibited by reduced pH_e, due to an increased monomer-dimer ratio [33]. Presently it is unknown if similar pH-dependent effects on stability of dimmers is seen with other isoforms of NOS. The iNOS inhibition under conditions of reduced pH_e is not universal and is tissue- and cell-specific. For instance, macrophages exhibit enhanced iNOS activity under these conditions and not surprisingly, this effect is associated with normal NADPH coupling [31]. These studies suggest that acidosis may impair nitric oxide production in the kidney and support the notion that acidosis may be one of the contributors to nitric oxide deficiency in CRF.

Endothelins (ETs)

Endothelin (ET), first described by Yanagisawa et al in 1988 [34], is a potent endothelial-derived vasoconstrictor as well as a sodium-regulating peptide. The ET system comprises of four endothelins (ET_{1-4}) and the receptors that mediate the biologic actions of ETs. In the kidney, ET_1 is synthesized in the endothelium of renal vasculature (both in macrovasculature and microvasculature), mesangial cells, peritubular capillaries and in the epithelium of the proximal tubule, mTAL [35] and the collecting duct, especially in inner medullary collecting duct (IMCD) [36]. The biologic effects of ETs are mediated by specific receptors. ET_A receptors are predominantly distributed in the vasculature of glomeruli and medulla while ET_B receptors are present in the renal tubules and collecting ducts [37]. ET_1 secretion is primarily regulated by transcription and partly by extracellular factors such as osmolality and humoral agents such as antidiuretic hormone. ETs are not only powerful vasoconstrictors but also mediate some nonhemodynamic renal functions [38].

Role of ETs in proximal tubular acidification mechanisms

Several lines of evidence incriminate ETs in the renal acidification mechanisms under physiologic conditions and in mediating renal responses initiated by metabolic acidosis [7, 38, 39, 40]. Chronic metabolic acidosis is

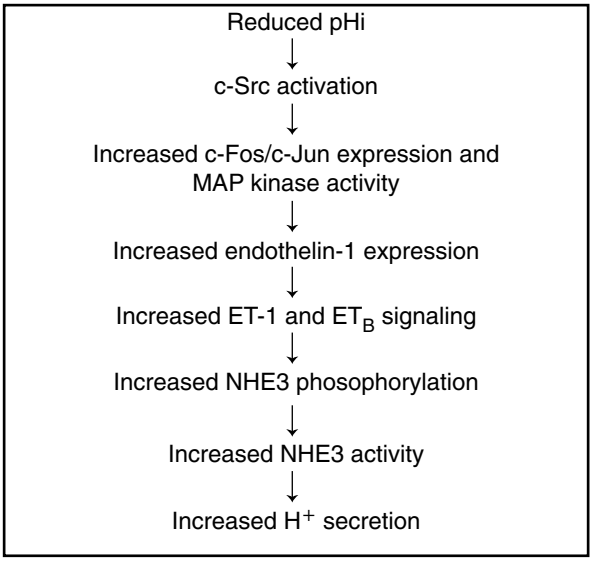

Fig. 3. Mechanisms of endothelin (ET)-mediated effects on H^+ secretion resulting from tissue acidosis.

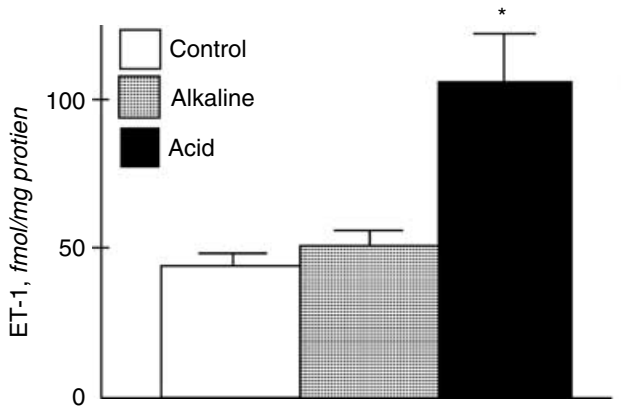

Fig. 4. Cell-associated endothlin (ET-1) concentrations in renal microvascular endothelial cells (RMVECs) after 12 hours of incubation in control (pH 7.2), alkaline (pH 7.4), and acidic (pH 7.0) media. $^*P <$ 0.05 vs. control. Reprinted from Wesson et al, *J Clin Invest* 101:578–583, 1998. Permission requested.

usually associated with reduction of both extracellular and intracellular pH. However, chronic potassium deficiency is associated with decreased intracellular pH with no change in pH_e. ETs, particularly ET_1, modulate the NHE-3 activity in the proximal tubule which in turn is responsible for the regulation of proximal tubular apical H^+ secretion. ET_1 increases the activity of renal cortical brush border Na^+-H^+ antiport activity and the basolateral Na^+-$3HCO_3^-$ transporter [41]. Recent studies by Laghamani, Preisig, and Alpern [7] demonstrated that in metabolic acidosis, NHE-3 activity is stimulated by ET_1 working through ET_B receptor. Furthermore, these investigators showed that ET_1/ET_B-stimulated NHE-3 activity was due to an increase in apical NHE-3 abundance, which resulted from an increased exocytotic insertion of NHE-3 into the apical membrane [39]. In this study, the authors also established that the stimulation of NHE-3 activity by decreased intracellular pH depended on activation of Pyk2, c-Src (nonreceptor tyrosine kinases) and increased expression of the immediate early genes, *c-Fos/cJun*. Regulation of *c-Fos/c-Jun* may involve mitogen-activated protein (MAP) kinase family (Fig. 3).

Role of ETs in distal tubular acidification mechanisms

ETs play an important role in renal acidification mechanisms in nephronal segments beyond the proximal tubule. For example, in the rat TALH, chronic metabolic acidosis increased mRNA and protein expression as well as the activity of NHE-3 [42]. The primary mechanism by which distal tubule contributes to acidification of urine is by titration of nonbicarbonate buffers. Reclamation of filtered bicarbonate that escapes proximal tubular reabsorption is an additional mechanism contributing to urinary acidification. Luminal H^+ secretion occurs in both processes and involves Na^+/H^+ exchange, H^+/K^+ ATPase and H^+-ATPase. In addition, Cl^-/HCO_3^- exchanger mediates bicarbonate secretion in the distal tubule. Augmented distal tubular acidification in response to dietary acid digestion is mediated by reduced bicarbonate secretion and increased H^+ secretion. Sustained changes in extracellular or intracellular acid-base parameters do not necessarily precede increased urinary acidification. Published studies evaluating the effect of ETs on distal acidification are very limited and were performed exclusively by Wesson and Dolson [40]. They demonstrated that intra-arterial infusion of ET_1 enhanced distal tubular acidification by increasing luminal H^+ secretion as well as by decreasing bicarbonate secretion. In addition, ET_1 secreted by renal microvascular ET cells mediated increased distal tubular acidification induced by dietary acid [43]. Furthermore, blockade of ET_{1B} and not ET_{1A} receptors blocked the tubular response of increased urinary acidification.

Effect of pH_e on ETs

The influence of pH_e on ET_1 secretion in the kidney was convincingly demonstrated by Wesson et al [43, 44]. Using human renal microvascular endothelial cells (RMVEC), these investigators showed that pH_e influenced the ET_1 produced by RMVEC. Acidic pH (7.0) stimulated ET_1 production compared to standard or control pH (7.2), while pH_a (7.4) had no effect on basal ET_1 production (Fig. 4). Thus, reduced pH_e enhanced ET_1 production by RMVEC, while under similar experimental conditions; ET_1 secretion was unaffected in macrovascular (aortic) endothelial cells. These data offer a potential explanation by which dietary acid could

enhance renal cortical ET_1 production, which is by increasing renal cortical acid content. The increase in renal cortical acid content leads to increased ET_1 levels in the interstitial fluid, which bathes both distal tubule epithelium and renal microvasculature possibly providing a paracrine communication between them. The authors suggest that since ET_1 increases fibronectin and collagen synthesis, the increased ET_1 levels resulting from a reduced renal mass may play a role in progressive renal sclerosis. The cellular microenvironment in renal failure, which includes acidosis, may affect the renal function through effects on ET_1 production.

However, as the authors acknowledged, the exact mechanisms by which lowered pH_e stimulated the ET_1 production was not clear from these studies [43, 44]. Specifically, they did not exclude the effect of lower bicarbonate on ET_1 production independent of effects from lower pH_e. Furthermore, kinases are known to modulate ET_1 production. For instance, protein kinase C modulates angiotensin II–induced ET_1 synthesis [45] and tyrosine kinase mediates insulin induced ET_1 synthesis [46]. Currently, it is unknown if kinases play any role in mediating the pH effects on ET_1 production. The observation that decreased pH_e in the studies of Wesson et al stimulated ET_1 synthesis in microvascular but not in macrovascular endothelial cells indicates differential sensitivity of ET_1 to pH_e in different cell types. These differences in pH sensitivity may relate to the magnitude of basal ET_1 secretion, and differences in the microenvironment that the endothelial cells are bathed in. Specifically, the basal pH of the medium that bathes the renal microvasculature is more acidic than that bathing the aortic endothelium [44]. Recent studies [47] indicate that chronic metabolic acidosis enhances the expression of pre-pro ET_1 in the kidney cortex, proximal tubule, and glomeruli and the increased expression correlates with the decrease in blood HCO_3.

In summary, ET_1 plays an important role in mediating the renal responses to systemic acid-base challenges and provides a mechanism for adaptive renal actions under physiologic and pathologic states. Changes in pH_e and acid-base status result in alterations in renal ETs that account for titration of renal acidification mechanisms both at proximal and distal tubular level. Furthermore, the up-regulation of ET_1 under acidic microenvironment may provide yet another mechanism for fibrosis and sclerosis, events that account for progressive renal functional deterioration that occurs in most forms of CRF [48].

ANGIOTENSIN

The kidney is both an important source of several components of the renin-angiotensin system as well as a target organ for their actions. Angiotensinogen is converted to angiotensin I by renin, and mRNA for both angiotensin and renin are found in juxtaglomerular cells and renal tubular cells. Angiotensin-converting enzyme (ACE), which cleaves angiotensin I to angiotensin II, is found principally in the lung but also in other tissues including the kidney. The principal effector of renin-angiotensin axis is angiotensin II, the biological actions of which are mediated through activation of at least two receptor subtypes, AT_1 and AT_2. Most angiotensin II effects are believed to be mediated by AT_1 receptors while AT_2 receptors are believed to be expressed only in fetal life although evidence is emerging in the recent literature for not only the expression but also for a functional role of AT_2 receptors in adult life. In addition to being a circulating hormone with strong vasoconstrictive effects in systemic and glomerular hemodynamics, angiotensin II has important paracrine effects on cell proliferation and tubular transport.

Role of angiotensin in renal acidification

Angiotensin II participates in the renal acidification mechanisms under normal conditions through several mechanisms. In the proximal tubule, angiotensin II stimulates Na-HCO_3 reabsorption by increasing the apical Na^+/H^+ antiporter activity (both through cAMP-dependent and cAMP-independent mechanisms) and also by stimulating the basolateral Na^+-$3HCO_3$ cotransporter activity. Using in vivo microperfusion of rat proximal convoluted tubule, Liu and Cogan [49] have first shown that angiotensin II increases proximal tubular bicarbonate reabsorption by a G1 protein–mediated inhibition of intracellular cAMP. However, the same group as well as others later had shown that protein kinase C activation without changes in intracellular cAMP levels may be involved in the proximal tubular bicarbonate reabsorption stimulated by angiotensin II [50, 51]. Studies in rabbit proximal tubular cells also confirmed that physiologic concentrations of angiotensin II stimulated amiloride-sensitive apical Na^+/H^+ antiporter activity [52]. Angiotensin II inhibited adenyl cyclase in renal cortical homogenates [53] and in cultured renal proximal tubular cells [54]. These studies suggested that angiotensin II stimulated the Na^+/H^+ antiporter by inhibition of adenyl cyclase, possibly through an inhibitory G protein. Schelling et al [55] showed that angiotensin II–stimulated sodium transport in proximal tubule was transduced by phospholipase C. These investigators showed that while angiotensin II inhibited adenyl cyclase, the effect of the latter on angiotensin II–mediated Na^+ transport was mediated by phospholipase C. Houillier et al [56] had shown that in intact proximal tubule cells, angiotensin II stimulated the apical Na^+/H^+ antiport through a G protein–dependent protein kinase C pathway, whereas at higher dose, angiotensin II inhibited Na^+/H^+ antiport activity through cytochrome

P-450–dependent metabolites. However, Cano et al [57] demonstrated that in OKP cells, angiotensin II increased Na^+/H^+ antiporter activity without affecting the cAMP production. Furthermore, the angiotensin II modulation of Na^+/H^+ antiporter activity may be dependent on the subtype of angiotensin II receptor [58]. In opossum kidney cells (OK-RR cells), for example, angiotensin inhibition by Dup 753, an AT_1 receptor blocker, abolished angiotensin II stimulation of Na^+/H^+ activity but not when PD123319, an AT_2 receptor blocker, was used. Thus, current evidence indicates that angiotensin II stimulates H^+ secretion by augmenting Na^+/H^+ activity either through cAMP-dependent or cAMP-independent pathways, although such effect may depend on the specific angiotensin II receptor subtype.

The nonreceptor tyrosine kinase, c-Src, plays a key role in the regulation of NHE-3 by acidosis in the proximal tubule and in mediating the effects of angiotensin II in the vascular smooth muscle cells. Tsuganezawa, Preisig, and Alpern [59] examined the role of c-Src in mediating the effects of angiotensin II–induced NHE-3 activation in cultured OKP cells. These studies confirmed that c-Src is required for the activation of NHE-3 by angiotensin II and thus plays an important role in mediating the effects of acidosis on Na^+/H^+ antiporter activity through angiotensin II. The effects of angiotensin II on NHE-3 may be biphasic and dose dependent [60].

The effects of angiotensin II on HCO_3^- transport in the loop of Henle are controversial. Studies by Good, George, and Wang [61] have demonstrated that angiotensin II inhibited HCO_3^- absorption in isolated perfused rat mTALH through a cytochrome P-450–dependent mechanism. However, more recent studies by Kwon et al [62] have demonstrated that NHE-3 may mediate enhanced HCO_3^- reabsorption in the mTAL in response to angiotensin II. In the collecting duct, intercalated cells secrete H^+ through increased expression of H^+-ATPase in the apical membrane in response to metabolic acidosis and thereby participate in acid-base homeostasis. Micropuncture studies showed that angiotensin II modulates renal net acid secretion in the distal tubule [63]. Recently Wall et al [64] have demonstrated that despite dramatic up-regulation of H^+ secretion in the distal tubule, angiotensin II infusion did not produce metabolic alkalosis. Compensatory reduction of H^+ secretion in other nephronal segments such as mTAL and cortical collecting duct was suggested as a possible explanation, since angiotensin II was shown to increase HCO_3^- secretion in the rabbit cortical collecting duct [65]. Wall et al [64] showed that the angiotensin II–induced reduction in $JtCO_2$ in outer medullary collecting duct (OMCD) in vitro was blocked by AT_1 blocker candesartan, indicating that such an effect is mediated by AT_1 receptors. These data strongly support the role of angiotensin in acid-base homeostasis.

EFFECT OF pH_e ON ANGIOTENSIN II

Acidosis stimulates renal ammonia production and secretion in the proximal tubule. Angiotensin II has been shown to stimulate renal ammoniagenesis in the proximal tubule. Studies by Nagami [66] examined the hypothesis that the effects of acidosis on ammonia production in the proximal tubule might be mediated through angiotensin II. Short-term infusion (18 hours) of ammonium chloride was used to induce metabolic acidosis in vivo mice and luminal ammonia secretion was measured in S_2 segments of proximal tubules microperfused in vitro. Higher rates of ammonia secretion were demonstrated in S_2 tubules from NH_4Cl-treated mice compared to control mice. However, the enhanced ammonia secretion was abolished when the animals received losartan, an angiotensin receptor blocker, along with NH_4Cl. Furthermore, addition of angiotensin II to the microperfusion solution enhanced the ammonia secretion in NH_4Cl-treated mice. The author concluded that a short-term acid challenge induced an adaptive increase in ammonia secretion by proximal tubule and that angiotensin II played an important role in the adaptive renal ammoniagenesis as a result of acute acidosis. On the other hand, studies in neonates suggested that the stimulation of renin-angiotensin axis by acute metabolic acidosis is negligible [67].

Angiotensin was also shown to mediate some of the renal effects of respiratory acidosis. The antinatriuretic response seen in acute hypercapnic acidosis could be blocked by angiotensin antagonists, suggesting the activation of renin-angiotensin system in response to acute hypercapnic acidosis [68]. Acute hypercapnic acidosis and hypoxemia causes vasoconstriction, possibly mediated by angiotensin II, stimulated by acidosis. However, studies that examined this hypothesis have not yielded consistent results. Studies in conscious dogs [69] subjected to combined acute hypoxemia and hypercapnic acidosis showed that angiotensin might not play a role in the initial renal vasoconstriction. However, angiotensin II attenuated the spontaneous recovery of renal hemodynamic variables to baseline as the acid-base disturbance continued and that vasopressin played no role in the initial intrarenal vasoconstriction [70]. In fact alpha-adrenoreceptors may play a more important role in mediating the renal vasoconstriction induced by metabolic acidosis than angiotensin II [71]. Furthermore, combined hypercapnic acidosis and hypoxemia act synergistically to activate rennin-angiotensin system [72] and play a major role in maintenance of renal hemodynamics under these conditions.

Current evidence suggests that metabolic acidosis stimulates renin-angiotensin axis and angiotensin II production [73]. Renal responses to metabolic acidosis exhibited in the collecting tubule may also involve mediation by angiotensin II. Recent studies [64] indicate that $JtCO_2$ was

reduced after NH_4Cl ingestion in OMCD in vitro, an effect believed to be mediated by angiotensin II suggesting that metabolic acidosis actually decreased H^+ secretion in collecting tubule, contrary to the expected compensatory response. The authors postulated that decreased H^+ secretion might augment K^+ absorption to attenuate hyperkalemia, seen in metabolic acidosis.

In summary, angiotensin may be involved in mediating several renal responses to both systemic metabolic and respiratory acidosis.

PROSTAGLANDINS

Prostaglandins are metabolites of arachidonic acid produced by the action of prostaglandin synthase or cyclooxygenase. Prostaglandins are produced in the kidney in various segments but the collecting duct accounts for major fraction of renal prostaglandin synthesis. ProstaglandinG_2 (PGG_2) and prostaglandin H_2 (PGH_2) are in general vasoconstrictive while prostaglandin E_2 (PGE_2) and prostaglandin I_2 (PGI_2) are vasodilatory. In addition to strong effects on vascular smooth muscle and mesangial cells, prostaglandins also play an important role in renin release and tubular salt and water transport.

Role of prostaglandins in renal acidification

Most of the available evidence indicates that the predominant effect of the prostaglandins on renal function involves salt and water transport. Currently, it is unclear if prostaglandins play any significant role in renal acidification under basal conditions. Prostaglandins inhibited renal ammoniagenesis in the rat proximal tubule [74]. Recent reports suggest that PGE_2 may stimulate organic anion transport (OAT) in the basolateral membrane of several models of proximal tubules, including OK cells, isolated renal S_2 rabbit proximal tubule segments and in human renal epithelial cells expressing OAT [75]. In vitro studies have shown that PGE_2 inhibits net HCO_3^- transport in the OMCD [76]. In Madin-Darby canine kidney (MDCK) cells, PGE_2 caused elevation of intracellular pH resulting in intracellular alkalinization by stimulation of the Na^+/H^+ exchanger [77]. In sickle cell disease, indomethacin, a prostaglandin inhibitor, decreased renal ammoniagenesis [78]. Low urinary levels of vasodilator prostaglandins have been reported in Gordon's syndrome [79], incriminating renal hypoprostaglandinism as a pathophysiologic mechanism. Gordon's syndrome is characterized by hypertension, hyperkalemia, and normal renal function with hyperchloremic metabolic acidosis. In these patients, levels of vasodilator prostaglandins PGE_2 and 6-keto-PGF_1-α are generally low.

Effects of pH_e on renal prostaglandins

Currently, there is very limited information about the effects of pH_e changes on the renal prostaglandin synthesis. Meclofenamate enhanced the renal ammoniagenesis induced by mild acute metabolic acidosis, which is associated with increased urinary excretion of PGE_2 and $PGF_{2\alpha}$ [80]. Based on these studies, it has been suggested that vasoactive prostaglandins may mediate renal acidification through other hormones such as angiotensin II and arginine vasopressin (AVP). Sahai, Goyal, and Tannen [81] investigated the mechanism of $PGF_{2\alpha}$-mediated inhibition of renal ammoniagenesis and demonstrated that under acidified conditions, $PGF_{2\alpha}$-stimulated Na^+/H^+ antiporter activity in porcine proximal tubular cell (LLC-PK1) cultures. $PGF_{2\alpha}$ in lower concentrations increased cellular alpha-ketoglutarate concentration, an effect that was lost at higher concentrations. Thus, prostaglandins, especially $PGF_{2\alpha}$ and PGE_2, modulate the renal ammoniagenesis stimulated by metabolic acidosis [82]. Tannen and Goyal [83] showed that in perfused kidneys, the urinary inhibitor of ammoniagenesis induced by acute respiratory acidosis was $PGF_{2\alpha}$. Indirect studies suggest that vasodilatory prostaglandins mediate some of the adverse renal effects of distal renal tubular acidosis such as hypercalciuria, kalliuresis, and hyperreninemia since indomethacin ameliorates these effects [84]. Jones et al [74] studied the effect of systemic acid-base disturbances on prostaglandin production in rat kidneys. Prostaglandins inhibit renal ammoniagenesis in rats. Acute metabolic acidosis increased total ammonia synthesis but also stimulated prostaglandin production. In these studies, prostaglandin inhibition with meclofenamate stimulated renal ammoniagenesis. In rats with mild metabolic acidosis, renal ammoniagenesis, and urinary excretion of PGE_2 and $PGF_{2\alpha}$ were greatly increased and administration of meclofenamate markedly reduced urinary prostaglandin excretion, further augmented ammonia synthesis, and significantly increased the fractional excretion of NH3. Inhibition of stimulated prostaglandin synthesis during severe metabolic acidosis did not increase ammoniagenesis or fractional excretion of NH3. Acute metabolic alkalosis did not alter production of prostaglandins or ammonia, but reduced the fractional excretion of NH3. It was concluded from these studies that prostaglandins inhibit ammonia synthesis in normal rats and in those undergoing mild metabolic acidosis. Severe acidosis overrides this inhibitory effect of prostaglandins on ammoniagenesis, whereas metabolic alkalosis suppresses the stimulatory effect of prostaglandin inhibition on ammoniagenesis.

CLINICAL RELEVANCE OF INTERACTIONS OF RENAL VASOACTIVE FACTORS AND pH_e

Chronic renal failure (CRF)

Metabolic acidosis is an integral feature of many forms of CRF. Since decreased intrarenal nitric oxide production is known to mediate mesangial sclerosis, a critical determinant of progressive CRF, it is important to

understand the effects of acidosis on intrarenal nitric oxide production. Studies from my laboratory [31] and others support that decreased pH_e is associated with nitric oxide inhibition. Based on these data, it is likely that inhibition of intrarenal nitric oxide synthesis may be much more pronounced in those forms of CRF which are associated with severe metabolic acidosis. Untreated acidosis under such conditions may contribute to the progression of CRF by amplifying the intrarenal nitric oxide inhibition that is seen in most forms of CRF. Further studies are needed to establish the role of nitric oxide inhibition due to acidosis in the progression of CRF and whether amelioration of acidosis enhances the intrarenal nitric oxide production and decreases the rate of progression of renal failure. Furthermore, some evidence exists to incriminate prostaglandins in mediating demineralization of bone, a process that may be associated with the metabolic acidosis of uremia [85].

Diabetic renal disease

Diabetic nephropathy itself is associated with severe abnormalities of nitric oxide with mechanisms operating in parallel that both enhance and diminish the nitric oxide release in the kidney [86]. Uncontrolled diabetes was shown to increase NOS activity particularly NOS I and NOS III and stimulate Na^+/H^+ exchange activity in proximal tubules [87]. However, several studies indicate that as the duration of diabetic nephropathy increases, factors that suppress bioavailability of nitric oxide such as formation of advanced glycation end-products (AGEs) and reactive oxygen species (ROS) prevail [88]. In addition, metabolic acidosis of renal disease may be severe and occur early in the course of diabetic renal failure. The problem is compounded by the frequent occurrence of type IV renal tubular acidosis in diabetic nephropathy.

Septicemia

There is a large body of evidence supporting the role of nitric oxide, particularly inducible nitric oxide, in the generation of metabolic acidosis of septicemia. Copious amounts of cytokine-mediated nitric oxide leads to vasodilatation and decreased tissue profusion and thereby result in lactic acidosis. Recent studies by Kellum et al [89] showed that moderate hyperchloremic metabolic acidosis (base excess -5 to -10 mEq/L) worsened hemodynamics in a rat model of sepsis, in association with increased stable metabolites of nitric oxide in plasma nitrites, and nitrates (NOx), while severe acidosis (base excess -10 to -15 mEq/L) also worsened hemodynamics but without affecting the plasma nitrites and nitrates. The authors concluded that the mechanism(s) responsible for hypotension in this setting are unclear. However, they are likely to be complex, and may involve increased nitric oxide release when acidosis is moderate and other mechanisms when more severe. Furthermore, the effect of acidosis on plasma NOx may also depend on the type of acid involved [90]. For example, hydrochloric acid as in hyperchloremic metabolic acidosis increased lipopolysaccharide (LPS)-induced NOx release from macrophages while lactic acid, as seen in lactic acidosis, did not. Many studies that used various inhibitors of NOS, particularly iNOS specific blockers, resulted in amelioration of acidosis in the context of septicemia [91]. However, many detrimental effects have been reported from such NOS inhibitors, including increase in pulmonary resistance, decreased cardiac output, and organ perfusion [92]. In the context of endotoxic shock, nitric oxide released from eNOS (NOS III), on the other hand, may have a beneficial effect in protecting against the deleterious effects of thromboxanes and leukotrienes [92]. Thus selective iNOS blockers are preferred over nonselective inhibitors for treatment of septic shock [93]. Studies have demonstrated that selective iNOS blockers are better than even adrenergic support with norepinephrine in treatment of sepsis [94]. In patients with sepsis, treatment with ibuprofen, an inhibitor of vasodilatory renal prostaglandins, reduces levels of prostacyclin and thromboxane and decreases fever, tachycardia, oxygen consumption, and lactic acidosis, but it does not prevent the development of shock or the acute respiratory distress syndrome and does not improve survival [95]. The pathogenic role of ET in endotoxic shock is supported by the efficacy of selective blockade of ET receptors, specifically ETB in diminishing liver injury and failure [96].

Diabetic ketoacidosis (DKA)

While there is a rapidly growing data about abnormalities of nitric oxide in diabetic state, literature about nitric oxide alterations in DKA is limited. Iori et al [97] reported increased levels of nitrate and iNOS mRNA and protein in lymphomonocytes in DKA. These changes are believed to mediate vascular abnormalities in DKA, such as increase in precapillary vasodilation. But correction of ketosis only partially decreased the changes in nitric oxide abnormalities and iNOS expression. The authors ascribed the persistence of nitric oxide abnormalities to large amounts of insulin required to correct ketosis. Urinary excretion of prostaglandins has been reported to be abnormal in patients with diabetic ketoacidosis [98]. Specifically vasodilatory prostaglandins such as $PGE_{2\alpha}$ and its metabolites as well as PGI_2, were increased in urine of patients with DKA. It was suggested that such changes may be protective and counter balance the vasoconstrictor processes associated with DKA.

Renal tubular acidosis

Knockout mice deficient in nNOS exhibit a metabolic acidosis [11], clearly indicating an important role for

nitric oxide in the transport of H^+/HCO_3^- in the kidney. Modulation of renal tubular acidosis (RTA) by nitric oxide has been recently described by Tsuruoka et al [99] in a rat model of cyclosporine A (CyA) toxicity. Indeed, CyA has been found to inhibit nitric oxide production in a variety of cell types [100]. Inhibition of nitric oxide synthesis may be one of the mechanisms underlying the CyA effect. Tsuruoka et al [99] demonstrated that administration of L-NAME, a NOS inhibitor, exacerbated and L-arginine ameliorated the impaired HCO_3 reabsorption seen with acid challenged CyA-treated rats. Using models of perfused cortical collecting duct, it was concluded that CyA causes distal RTA by inhibiting H^+ pumps in the distal nephron.

CONCLUSION

It is apparent from the preceding discussion that changes in systemic acid-base status regulate the generation of vasoactive cytokines in the kidney and these factors, in turn, may play a role in the adaptive renal responses to the initiating alteration in the H^+ concentration in the tissues. Furthermore, the data presented strongly support the concept that these factors, particularly ET and nitric oxide, play an important role in the normal H^+ homeostasis by regulating renal acidification mechanisms. In general, the currently available information suggests an inverse relationship between nitric oxide and ET, with reference to their effects on renal acidification mechanisms as well as pH_e effects on these factors. Thus, reduced pH_e inhibits nitric oxide while stimulating ETs and possibly even angiotensin II in the kidney. Further studies need to be conducted to enhance the current understanding of the interactions of angiotensin II and prostaglandins with renal acidification mechanisms in health and disease. Such studies are necessary to unravel the complex interactions between the H^+ concentration and the vasoactive cytokines and to expand our knowledge of regulatory controls involved in the homeostatic mechanisms in conditions of health and systemic acid-base disorders.

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