Endothelin Role in Kidney Acidification

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Endothelin is a potent vasoconstrictor that recent studies show modulates transport in kidney tubules, including that related to acidification. The data support a physiologic role for endothelin in mediating enhanced kidney tubule acidification in response to an acid challenge to systemic acid-base balance status. The data to date do not support an endothelin role in maintaining kidney tubule acidification in control, nonacid-challenged states. Endothelin also contributes to the enhanced acidification of some pathophysiologic states and might have a role in some of the untoward outcomes associated with these conditions. This review supports continuation of studies into the physiologic and possibly pathophysiologic role of endothelin in settings of increased tubule acidification.

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Enhanced net acid excretion by the kidney contributes to correcting the disturbance in systemic acid-base status induced by an acid challenge. Because a systemic acid challenge increases kidney endothelin activity and endothelin increases acidification in kidney tissue in vitro, investigators have explored a possible physiologic role for endothelin in mediating kidney acidification. These studies described herein support a physiologically important role for endothelin in mediating enhanced kidney acidification that occurs in response to a systemic acid-base challenge. The data suggest that endothelin also contributes to increased kidney acidification observed in pathophysiologic states such as chronic metabolic alkalosis and chronic kidney disease (CKD). Improved understanding of the cascade of factors that contribute to enhanced kidney acidification in response to an acid challenge and how these factors lead to increased acidification will help in the design of better treatment strategies for disturbances of acid-base balance. This understanding also should enhance our understanding of pathologic changes associated with pathophysiologic states of chronically increased kidney acidification such as CKD, to which increased endothelin activity might contribute.

Overview of Endothelin Biology

Endothelins (ET) are a family of 21 amino acid peptides known best as powerful vasoconstrictors. ET-1 is the major isoform of those described (ET-1, ET-2, and ET-3) and is the only one expressed as a protein in human kidneys. Its production is regulated at the level of transcription in endothelin-producing tissues examined, including kidney microvascular endothelium. Unlike other vasoactive substances, endothelin does not accumulate in secretory granules but is synthesized and released constitutively and/or in response to a stimulus. The initial gene product is the 212 amino acid peptide prepro-ET-1 that is converted to a 38 amino acid peptide called big ET-1 by ET-converting enzyme. Although many enzymes convert big ET-1 to ET-1, the enzymes that mostly do so at physiologic pH (optimum pH, 7.0) are the family of neutral metalloproteinases. In turn, this locally produced ET-1 is degraded by neutral endopeptidase. Many tissues have both the synthetic and degradation machinery for ET-1 including kidneys, suggesting local production and degradation of ET-1 that modulates tissue function in an autocrine/paracrine fashion (see later).

Overview of the Kidney Response to Acid-Base Challenges

Because altered systemic acid-base homeostasis adversely affects cell function, multiple systems coordinate to help maintained body fluid hydrogen ion concentration ([H^+]i) within a
range in which cells function optimally. Metabolically pro-
duced H⁺ might increase body fluid [H⁺] and induce a phys-
ologic response designed to return [H⁺] to the optimal
range. Two responses intended to maintain and/or restore
optimal range of body fluid [H⁺] are (1) H⁺ buffering,¹⁷ in
which body buffers bind added H⁺ to minimize the [H⁺] in-
crease that would otherwise occur, and (2) H⁺ excretion,¹⁸ in
which H⁺ is removed from the body. Because H⁺-trated
buffers less effectively buffer subsequently added H⁺, buffer-
bound H⁺ eventually must be excreted to regenerate body
buffers and restore buffering capacity. Consequently, all
added H⁺ eventually must be excreted to restore acid-base
homeostasis to that before H⁺ addition. Indeed, net H⁺ ex-
cretion in human beings is equivalent to net H⁺ production
in the steady state and the kidney is the major route of H⁺
excretion.¹⁸,¹⁹ These data support the importance of explor-
ing kidney mechanisms by which H⁺ excretion increases in
response to an H⁺ challenge.

Most experimental models exploring the effect of a sys-
temic challenge to acid-base status have used acid challenges
that are in marked excess of that routinely encountered by
animals or human beings.²⁰ Such studies show an important
role for enhanced acidification in the proximal tubule in me-
diating the kidney response to excrete administered acid.²⁰
More modest acid challenges induced by chronic dietary acid
intake, however, yield observable increases in distal nephron
acidification with little to no observable increase in proximal
nephron acidification.²¹ This suggests that a greater propor-
tional increase (compared with the respective steady-state) in
distal rather than proximal nephron acidification mediates
enhanced net acid excretion in response to these more phys-
ologic challenges to systemic acid-base status. Enhanced dis-
tal nephron acidification in response to these more physio-
logic acid challenges is mediated by both decreased HCO₃⁻
secretion and increased proton (H⁺) secretion.²¹

More physiologic acid challenges to systemic acid-base sta-
tus lead to marked increases in urine net acid excretion with
a measurable increase in distal nephron acidification but
these changes are associated with little to no measurable
changes in plasma acid-base parameters.²¹ These data ques-
tion whether mediators of a sustained increase in kidney
acidification require sustained changes in plasma acid-base
parameters. Alternatively, other sensors, possibly intracellu-
lar, might be sufficient to maintain the necessary cascade of
responses that sustain an increase in kidney acidification. In
vitro studies show that intracellular pH of cultured kidney
epithelial cells chronically exposed to acid media for 48
hours was not different from control yet these cells had in-
creased Na⁺/H⁺ antiporter activity.²² In vivo studies show
that a chronic dietary acid challenge that increases kidney net
acid excretion and distal nephron acidification without meas-
urable changes in plasma acid-base parameters nevertheless
increases acid content of the kidney interstitium,²³ a commu-
nicating space between vascular endothelium and tubule ep-
ithelium.²⁴ These data suggest that persistent, measurable
alterations in plasma or intracellular fluid acid-base param-
eters are not necessary to permit a sustained increase in kidney
tubule acidification. These data further suggest that parame-
ters of increased body acid content other than plasma or
intracellular acid-base parameters serve as sensors that lead
to enhanced kidney acidification. Possible acid sensors in-
clude activated intracellular systems such as c-SRC, ERK,²⁵
and Pyk₂.²⁶ Activation of these intracellular systems impor-
tantly leads to endothelin-mediated upregulation of Na⁺/H⁺
exchange activity in proximal tubule epithelia in vitro.²⁴,²⁶
Consequently, chronic acid challenges might increase kidney
acidification through altering intracellular systems that lead
to increased production of substances that directly influence
components of kidney acidification. Increased endothelin ac-
divity might be one such mechanism that is stimulated by an
acid challenge and that mediates increased kidney acidifica-
tion in this setting.

Endothelin Production
by Vascular Endothelium

Endothelin production by human vascular endothelium occurs
in arterial macrovascular,²⁷,²⁸ arterial microvascular,¹⁰,²⁸ and
venous²⁹,³⁰ endothelium. Secretion of endothelin from vascular
endothelial cells is predominantly toward their basolateral sur-
faces,²⁹ suggesting paracrine modulation by endothelin in tis-
ues with which vasculature is associated, as suggested earlier.
There also is evidence for autocrine regulation of cellular endo-
thelin secretion by vascular endothelium.³¹,³²

Endothelin Production
by Kidney Parenchyma and
Its Possible Physiologic Role

Endothelin is produced by both glomerular and tubule kid-
ney cells. In addition to the previously indicated microvas-
cular endothelium of the glomerular capillary tuft endothelin
production, mesangial³¹ and glomerular epithelial cells³² pro-
duce and secrete endothelin. Kidney tubule epithelia that
produce and secrete endothelin in vitro include the proximal
tubule,³³ medullary thick ascending limb,³⁰ distal tubule,³⁷
 cortical collecting tubule,³⁸ and inner medullary collecting
duct,³⁹ with the greatest amount coming from the latter.³⁶
Kidney tubule epithelia have endothelin receptors, predom-
nantly of the B subtype,⁴⁰ located predominantly on basolat-
eral surfaces.⁴¹ In addition, the kidney has a system for local
endothelin degradation,¹⁴,¹⁵ consistent with paracrine⁴ and/or
autocrine⁴ control of kidney tubule function by locally pro-
duced endothelin. Because of the intimate relationship be-
tween kidney vasculature and tubules²⁹ and because the
interstitial space between them contains endothelin,³ this anat-
omy permits paracrine control of kidney tubule function, pos-
sibly by endothelin secreted by vascular endothelium.³,¹⁰

Endothelin Production by Adrenal
Cortex and Its Possible Role in
Enhanced Kidney Acidification

The adrenal cortex synthesizes endothelins⁴² that stimulate
secretion of both glucocorticoids and mineralocorticoids.⁴³,⁴⁴
Because glucocorticoids$^{15,46}$ and mineralocorticoids$^{47}$ stimulate H$^+$ secretion in kidney tubule epithelium, adrenal cortical endothelins might increase kidney acidification indirectly and thereby contribute to the kidney response to an acid challenge.

**Effects of Acid Challenge on Kidney Endothelin Production**

Chronic acid loading with dietary ammonium salts increases kidney expression of endothelin messenger RNA$^{48}$ and kidney production of endothelin.$^3$ Similarly, increased intake of acid-producing dietary protein induces increased expression of kidney endothelin messenger RNA and increased kidney endothelin production.$^{49}$ Furthermore, dietary acid$^{48}$ and increased intake of acid-producing dietary protein$^{51}$ each increase plasma aldosterone levels. In addition, an acid extracellular environment increases aldosterone secretion by adrenocortical cells in vitro.$^{34}$ If dietary acid challenges increase adrenal corticai endothelin production as it does in the kidney,$^3$ these data suggest that adrenal endothelin indirectly contributes to enhanced kidney acidification in this setting.

**Endothelin Effects on Acidification**

**Cells**

Endothelin increases intracellular pH of many cell types in vitro including skin fibroblasts,$^{53}$ platelets,$^{54}$ vascular smooth muscle,$^{35}$ glomerular mesangial,$^{36}$ cardiac myocyte,$^{37}$ and kidney epithelial cells.$^{38}$ The Na$^+/H^+$ antiporter is the cell membrane H$^+$ transporter that is influenced most consistently by endothelin in these indicated cell types. Endothelin increases Na$^+/H^+$ antiporter activity in kidney epithelial membrane vesicles$^3$ and cortical slices.$^6$

**Kidney Tubules**

In addition to enhancing H$^+$ transport across cell membranes, endothelin also influences acidification across kidney tubule epithelia. Endothelin mediates enhanced proximal tubule acidification associated with chronic metabolic acidosis induced by NH$_4$Cl loading and does so through stimulation of endothelin B-type receptors.$^{48}$ In these studies,$^{38}$ endothelin did not appear to mediate proximal tubule acidification in control, nonacid-loaded animals. Endothelin mediates the enhanced distal nephron acidification associated with chronic acid loading performed by dietary ammonium salts$^3$ and acid-producing dietary protein.$^{49,50}$ In addition, exogenous endothelin stimulates distal nephron acidification in vivo.$^{60}$ As observed in the proximal tubule, endothelin appears not to contribute to basal distal nephron acidification in control animals.$^{39,51}$ In the loop of Henle, endothelin stimulates local nitric oxide (NO) release$^{61}$ and NO inhibits thick ascending limb Na$^+/H^+$ exchange,$^{62}$ suggesting that endothelin indirectly inhibits acidification in this nephron segment. Because endothelin-induced NO action on thick ascending limb Na$^+/H^+$ exchange activity might more importantly inhibit NaCl reabsorption in this nephron segment,$^{63}$ this indirect action of endothelin action in the thick ascending limb would increase Na$^+$ delivery to the distal nephron with an anticipated increase in distal nephron Na$^+$/H$^+$ exchange activity.$^{64}$ Enhancing distal nephron acidification. Consequently, the net effect of endothelin is increased proximal tubule and distal nephron acidification.

**Direct Mechanisms by Which Acid-Induced Endothelin Affects Kidney Acidification**

Endothelin enhances Na$^+/H^+$ exchange activity in many cell types in vitro as indicated earlier, most of which express primarily the Na$^+/H^+$ exchanger type 1 (NHE1) isoform.$^{63}$ In kidney proximal tubule, endothelin increases acidification in acid-challenged animals and cells primarily, if not exclusively, through enhanced activity of NHE3,$^1,66$ the major H$^+$ transporter in the proximal tubule. Because endothelin increases Na$^+/H^+$ exchange activity in the distal tubule and NHE2 appears to be the major apical isoform in this nephron segment,$^{68}$ endothelin appears to increase NHE2 activity. Although endothelin appears also to increase H$^+$-adenosine triphosphatase (ATPase) activity in the distal nephron,$^{49,59}$ no data exist to support the theory that endothelin directly stimulates this transporter in vitro. Instead, endothelin-induced stimulation of aldosterone secretion that in turn increases distal nephron H$^+$-ATPase activity appears to be the mechanism by which endothelin-receptor antagonism leads to reduced distal nephron H$^+$-ATPase activity.$^{39}$

**Possible Indirect Mechanisms by Which Endothelin Increases Kidney Acidification**

Because many cytokines influence distal nephron acidification and endothelin influences levels of many of these cytokines, endothelin might affect kidney acidification indirectly as well as directly. As discussed earlier, increased endothelin activity increases distal nephron H$^+$-ATPase activity through stimulated aldosterone secretion.$^{39}$ Of the 3 major distal nephron H$^+$ transporters (Na$^+/H^+$ exchanger, H$^+$-ATPase, and H$^+$, K$^+$-ATPase),$^{69}$ increased dietary acid leads to endothelin-mediated stimulation of 2 (Na$^+/H^+$ exchanger and H$^+$-ATPase).$^{49,50}$ Dietary acid-induced, endothelin-mediated, distal nephron acidification appears not to include stimulated H$^+$, K$^+$-ATPase activity.$^{49,50}$ Although K$^+$ depletion increases H$^+$, K$^+$-ATPase activity,$^{70}$ stimulated H$^+$, K$^+$-ATPase likely is not mediated by endothelin in chronic metabolic alkalosis associated with K$^+$ depletion.$^7$ In addition, endothelins might indirectly enhance kidney acidification through endothelin-stimulated secretion of glucocorticoids and mineralocorticoids by the adrenal cortex,$^{33,44}$ which stimulate Na$^+/H^+$ exchange$^{45,46}$ and H$^+$-ATPase,$^{47}$ respectively, in kidney tubules, as indicated earlier.
Reduced HCO₃ secretion contributes importantly to enhanced acidification induced by chronic dietary ingestion of mineral acid³,²¹ and acid-producing dietary protein.⁴⁹,⁵⁹ The resulting reduced HCO₃ delivery to the terminal distal nephron itself increases net acid excretion but this phenomenon also enhances NH₄⁺ secretion⁷¹ and permits secreted H⁺ to titrate non-HCO₃ buffers and thereby constitute net acid excretion rather than HCO₃ recovery.⁵⁰ Endothelin reduces distal nephron HCO₃ secretion (which reduces distal nephron acidification)³,⁴⁹,⁵⁹ indirectly through stimulated NO production.⁷² Other studies show that NO stimulates overall acidification in both the proximal⁷³ and distal⁷⁴ nephron in other settings.

Figure 1 outlines a proposed cascade by which endothelin mediates enhanced kidney acidification in response to an acid challenge. Reprinted with permission from Wesson et al.⁷²

**Possible Endothelin Role in Complications of Pathophysiologic Conditions Associated With Enhanced Kidney Acidification**

Animals with experimental models of chronic metabolic alkalosis develop fibrosis with subsequent calcium deposition in kidney parenchyma.⁸³ In addition, experimental animals with reduced kidney mass have augmented urine net acid excretion and increased glomerular filtration rate, consistent with enhanced distal nephron acidification.⁷⁹ Indeed, animals with reduced kidney mass have increased proximal⁸⁰ and distal⁸¹,⁸² nephron acidification in vivo. In addition, animals with CKD as a result of reduced kidney mass have increased urine ET-1 excretion, consistent with increased endogenous kidney ET-1 production.³ Endothelins mediate the enhanced distal nephron acidification in remnant kidneys,⁸⁴ an experimental model of CKD.

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