Angiotensin II: A Powerful Controller of Sodium Transport in the Early Proximal Tubule

Martin G. Cogan

Angiotensin II has recently been shown to exert potent control over sodium and water absorption in the proximal convoluted tubule. This transport stimulation is effected by receptors on both the luminal and basolateral membranes of cells located predominantly in the early, S1 proximal tubule. Angiotensin II increases transport primarily by a G protein-mediated reduction in intracellular cyclic adenosine monophosphate, which enhances the affinity of the Na⁺-H⁺ antiporter. Change in early proximal acidification ultimately causes alteration in the amount of sodium chloride leaving the proximal tubule and entering the urine. These direct tubular transport actions by angiotensin II may participate importantly in various physiological actions of the kidney, including the renal response to change in dietary sodium intake and in extracellular volume, as well as in pathophysiological processes such as hypertension. (Hypertension 1990;15:451–458)

Angiotensin II takes part in cardiovascular function by sensitively controlling vascular smooth muscle tone, mineralocorticoid biosynthesis, neural catecholamine release, and water metabolism. We now recognize that angiotensin II also participates in cardiovascular homeostasis by potent regulation of transepithelial sodium transport in renal tubular cells.1,2

An early inference that angiotensin II directly affects proximal tubule transport was furnished by Harris and Young.3 Microvascular injection of angiotensin II increased steady-state sodium concentration gradient in stationary fluid droplets within the late proximal convoluted tubule (PCT) of the rat. Schuster et al4 provided more definitive evidence regarding direct effects on transepithelial sodium transport in the late PCT. Angiotensin II (10⁻¹¹ and 10⁻¹⁰ M) increased sodium and volume absorption in the microperfused, subcortical rabbit PCT. Higher angiotensin II concentrations (10⁻⁸ to 10⁻⁶ M) inhibited sodium transport.3,4 However, angiotensin II-induced transport stimulation observed by Schuster et al4 was very small. The maximal response was only 16% above the baseline value and was trivial (0.1 nl/mm · min) compared with the normal amount of fluid reabsorbed by the PCT in vivo (18 nl/min).5

The studies described above were performed in the late (S2) subsegment of the PCT. With recent adaptation in micropuncture techniques, it is now recognized that the early (S1) PCT subsegment of the rat has a far more robust capacity for both active and passive transport than the S2 PCT, or for that matter, any other nephron segment.5,6 Indeed, although the S1 PCT is only a little over 1 mm in length in the rat, it is normally responsible for reabsorbing fully 20% of the solute and water that is filtered by the kidney.5 Were angiotensin II to control this powerful S1 PCT transport system, it would obviously have substantial potential for regulating renal sodium and water reabsorption.

As shown in Figure 1, angiotensin II does indeed have a potent effect on S1 PCT sodium and water transport in the Munich-Wistar rat.7–9 Angiotensin II infusion (20 ng/kg · min i.v.), which achieves a subpressor, systemic concentration within the physiological range (10⁻¹² to 10⁻¹¹ M), augmented S1 PCT water absorption by 2.5 nl/mm · min (closed squares) when measured by in vivo microperfusion.7 Inhibition of endogenous angiotensin II by saralasin (1 μg/kg · min i.v.) had the opposite effect, depressing volume absorption by −2.0 nl/mm · min (closed hexagons). Both angiotensin II and saralasin had lesser effects in the S2 PCT (±1 nl/mm · min) (open symbols).7 These direct tubular effects of angiotensin

From the Division of Nephrology and Cardiovascular Research Institute, University of California, San Francisco, and Nephrology Section, Veterans Administration Hospital, San Francisco, California.

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Address for reprints: Martin G. Cogan, MD, Nephrology Section (111J), Veterans Administration Medical Center, 4150 Clement Street, San Francisco, CA 94121.
II were exerted independently of changes in renal or systemic hemodynamics.

The full range of $S_1$ PCT transport affected by angiotensin II and saralasin (4.5 nl/mm·min) implied that angiotensin II could potentially regulate almost half of the sodium and water transport normally effected by this nephron segment. Summing the large $S_1$ and smaller $S_2$ PCT transport effects, angiotensin II had the potential of directly controlling 15% or more of all sodium reabsorbed by the kidney.

The following sections summarize recent advances in our understanding of the powerful regulation of $S_1$ PCT transport by angiotensin II in vivo. Effects of angiotensin II will be considered in ascending biological complexity, starting from actions on the cellular level, to the impact on solute and water transport when the whole PCT is considered as the sum of its $S_1$ and $S_2$ parts, and finally to integrated changes in whole nephron filtration-reabsorptive dynamics with implications for possible physiological and pathophysiological regulation of renal function in vivo.

**Cellular Actions of Angiotensin II in the Proximal Tubule**

**Solute Specificity**

Eighty five percent of reabsorbed sodium in the PCT is transported with either bicarbonate or chloride. Angiotensin II predominantly affects sodium bicarbonate transport. In the $S_1$ PCT, angiotensin II was found to increase bicarbonate absorption by more than 150 peq/mm·min. Inhibition of endogenous angiotensin II activity with saralasin had the opposite effect (~150 peq/mm·min).

The overall bicarbonate absorptive rate affected by angiotensin II and saralasin (~300 peq/mm·min) represents 60% of the acidification capacity of the early PCT (500 peq/mm·min). The $S_1$ PCT is normally responsible for reabsorbing half of the filtered bicarbonate load. Thus, angiotensin II possessed the capability of regulating fully 30% of all renal acidification. Quantitatively, the control of hydrogen ion secretion by angiotensin II in the early proximal nephron is 1–2 orders of magnitude greater than that by aldosterone in the distal nephron.

Angiotensin II and saralasin infusions cause lesser change in bicarbonate absorption in the $S_1$ PCT ($±28$ peq/mm·min) and in chloride absorption in both the $S_1$ and $S_2$ PCT ($±100$ peq/mm·min). As will be discussed more fully below, angiotensin II-induced change in sodium chloride transport may be at least partially due to alteration in hydrogen ion secretion and nerve activity and of great physiological significance with respect to overall tubule electrolyte handling.

**Modes of Action**

Angiotensin II can affect epithelial cell function directly, by occupancy of receptors on the cell membrane, or indirectly, by presynaptic receptors that control catecholamine release from nerves having terminals on the cell. Angiotensin II primarily controls sodium transport in the intestine by this latter, neurally dependent mechanism. PCT cells have angiotensin II receptors and are richly innervated, potentially enabling both means of transport regulation.

After denervation of the PCT, angiotensin II can still markedly augment bicarbonate absorption, but effects on sodium chloride absorption are greatly diminished. Thus, angiotensin modulation of sodium bicarbonate absorption in the PCT occurs via epithelial cell receptors, whereas change in sodium chloride absorption is predominantly via presynaptic receptors on renal nerves.

The more sensitive transport regulation by angiotensin II in the $S_1$ than in $S_2$ PCT segments (Figure 1) predicts that greater receptor density exists on $S_1$ PCT cells. As shown in Figure 2 in microdissected segments in vitro, specific $[^{125}I]$angiotensin II binding is more than 10-fold higher in the $S_1$ PCT compared with the mid and late $S_2$ PCT (4,000 vs. 300–500 amol/cm). Half-maximal binding concentration (5–6 nM) is similar in both segments. Thus, the axial heterogeneity of angiotensin II receptor density on $S_1$ compared with $S_2$ cells (Figure 2) explains the gradation in acidification response (Figure 1).

**Polarity of Response**

Using techniques to differentially separate cell membranes, Douglas showed that angiotensin II receptors exist on both the luminal and basolateral membranes. However, the functional importance of these receptors was not established until recently. Angiotensin II, perfused into the lumen at physiological concentrations (10$^{-10}$ and 10$^{-11}$ M), significantly augments early PCT bicarbonate absorption as shown in Figure 3 (middle two bars). The signaling-transport responses evoked by angiotensin II binding to the luminal receptor are only 30–50% of those
observed when angiotensin II occupies the basolateral receptor after intravenous infusion of angiotensin II when the luminal perfusate is devoid of angiotensin II (Figure 3, right bar). Nevertheless, angiotensin II is unique among renal hormones in being able to elicit a change in transepithelial transport from both the luminal and basolateral aspects of the cell.

The bipolarity of angiotensin II signaling raises the possibility that either luminal or basolateral receptors may physiologically operate to control S1: epithelial cell function under normal conditions. Angiotensin II is freely filtered by the glomerulus and thereby gains access to luminal receptors (before degradation by proximal brush border proteolytic enzymes and endocytotic mechanisms) in addition to access to basolateral receptors through the peritubular capillaries and interstitium. Either or both modes of hormone delivery could conceivably affect early PCT transport. Another intriguing possibility is that angiotensin II is actually produced in the PCT cell itself. The PCT has messenger RNA for angiotensinogen, can endocytose filtered or interstitially delivered renin or use another resident aspartyl protease (e.g., cathepsin) to form angiotensin I, and has a rich supply of converting enzyme to allow transformation to angiotensin II. The possibility of conversion of angiotensin I to angiotensin II has been supported by peritubular capillary infusion studies. Thus, autocrine, paracrine, or endocrine modes of control of PCT function by angiotensin I and II are all plausible and quite interesting possibilities requiring further in vivo study.

**Mechanism and Kinetics**

Net sodium bicarbonate absorption in the PCT is the sum of active hydrogen ion secretion and passive bicarbonate back leak. Interestingly, angiotensin II markedly reduces paracellular bicarbonate permeability in the S1 and S2 PCT by about 40%, perhaps by changing junctional complex structure due to altered cell shape or cytoarchitecture. However, the quantitative impact incurred by this change in permeability on back leak and hence net bicarbonate absorption is minor.

The major change in bicarbonate absorption induced by angiotensin II is on the active component, cellular hydrogen ion secretion. The Na+-H+ antiporter is the principal luminal mechanism effecting hydrogen ion secretion in S1 and S2 PCT cells. Amiloride (4 mM), an effective inhibitor of Na+-H+ antiporter activity, abolishes more than 80% of the angiotensin II–induced stimulation of PCT hydrogen ion secretion in vivo and in vitro. Thus, angiotensin II augments PCT transport by enhancing activity of the Na+-H+ antiporter or a transporter in series with it.

Kinetic analysis in vivo reveals that angiotensin II has no effect on the maximum velocity (Vmax) but rather reduces the apparent Km of the Na+-H+ antiporter. Similar kinetics have been observed in vitro for angiotensin II regulation of the Na+-H+ antiporter in vascular smooth muscle cells.

**Second Messengers**

A signaling system used by angiotensin II in many cells, including PCT cells, involves reduction of adenylyl cyclase activity. Reduction in cyclic adenosine monophosphate (cAMP) in S1 PCT cells stimulates bicarbonate transport because cAMP inhibits Na+-H+ antiporter activity. cAMP is transported with first-order kinetics out of all metazoan cells, including PCT cells, so that extracellular cAMP egression into the tubular fluid reflects intracellular cAMP concentration. Stimulation of S1 PCT bicarbonate absorption by angiotensin II is associated with a significant decrease in tubular fluid cAMP delivery (18±2 to 12±2 fmol/mn·min). Parathyroid hormone (PTH) elicits the expected opposite biochemical and physiological effects. As shown in Figure 4, over a wide range of
angiotensin II and PTH activities, bicarbonate absorption correlates inversely with tubular fluid cAMP \((r = -0.86, p < 0.001)\). The span of \(S_i\) PCT bicarbonate absorption potentially regulated by intracellular cAMP is from 100 to 600 \(\mu\)eq/mm • min, representing 80% or more of the \(S_i\) PCT capacity and hence virtually half of total renal acidification.

Pertussis toxin pretreatment in vivo significantly attenuates (by 35-45%) the angiotensin-induced increase in bicarbonate absorption and decrease in cAMP delivery, indicating Gi protein intermedation. Clamping of intracellular cAMP concentration by luminal perfusion with dibutyryl cAMP \((10^{-5} \text{ M})\) abolishes the transport response to angiotensin II. These findings are consistent with a model shown in the right side of Figure 5 in which angiotensin II binding to its receptor causes a Gi protein–coupled inhibition of adenylate cyclase, which diminishes intracellular cyclic adenosine monophosphate (cAMP) concentration and protein kinase A activity with consequent stimulation of the \(Na^+\)–\(H^+\) exchanger.

As occurs in other cells, angiotensin II probably uses multiple signaling pathways in the PCT. Although angiotensin II may affect the intracellular cyclic guanosine monophosphate (cGMP) level in hepatocytes, it does not do so in the PCT. There is no cGMP egression from PCT cells under the influence of endogenous angiotensin II, and exogenous administration of a permeable analogue of cGMP has no effect on PCT transport. A signal-transduction pathway possibly used by angiotensin II is phosphatidylinositol breakdown with subsequent increase in protein kinase C and intracellular calcium concentration. When protein kinase C or intracellular calcium are rendered incapable of responding to hormonal activation in the \(S_i\) PCT (using phorbol ester, sphingosine, or calcium ionophore), transport stimulation of angiotensin II is attenuated by approximately one third. Protein kinase C activation does not interfere with cAMP levels or hormonal response in the PCT. Thus, as shown in the left hand side of Figure 5, protein kinase C or intracellular calcium may independently mediate as much as a third of the acidification response to angiotensin II in vivo. Nevertheless, the major signaling mode used by angiotensin II, which is responsible for at least two thirds of the physiological transport effect, remains reduction in cAMP.

**Hormonal Interactions**

Angiotensin II has actions that are opposite in direction to but that are additive to those of PTH.
Ion Secretion in Sodium Chloride Transport

bicarbonate reabsorption. Mechanisms by which role of early proximal convoluted tubule hydrogen ion secretion in sodium chloride transport when the PCT is considered as a whole and thus adjusts extracellular volume.

A recent report using shrinking split-droplets suggested atrial natriuretic factor (ANF) could antagonize angiotensin II stimulation of PCT transport. This finding was not confirmed when the more reliable technique of in vivo microperfusion was used. ANF does not affect basal or angiotensin II-stimulated bicarbonate, chloride, or water absorption in either the S₁ or S₂ PCT. These results may not be surprising because the PCT lacks ANF receptors, participate guanulate cyclase, or the ability to generate the second messenger of ANF cGMP.

Physiological Impact of Angiotensin II on Sodium Chloride Reabsorption in the Proximal Tubule

Although a powerful regulator of hydrogen ion secretion in the early PCT, it should not be assumed that angiotensin II necessarily alters acid base balance under normal conditions. On the contrary, we have advanced the thesis that angiotensin II controls sodium chloride reabsorption when the PCT is considered as a whole and thus adjusts extracellular volume.

Role of Early Proximal Convoluted Tubule Hydrogen Ion Secretion in Sodium Chloride Transport

Under free-flow conditions, we have proposed that the effect of angiotensin II on early PCT acidification is physiologically "translated" to alter net sodium chloride reabsorption in the whole PCT. Two considerations are important for understanding the basis of this proposal. First, in the S₁ PCT hydrogen ion secretion effects sodium chloride as well as sodium bicarbonate reabsorption. Mechanisms by which Na⁺-H⁺ exchange accomplishes sodium chloride reabsorption include: direct transcellular means by parallel exchangers (Na⁺-H⁺ and Cl⁻-Base⁻) and indirect means for allowing paracellular diffusive flux by reducing the luminal bicarbonate concentration and thus raising the luminal chloride concentration. Second, in the S₁ PCT excellent intrinsic load-dependent transport normally exists for sodium bicarbonate but not for sodium chloride.

Angiotensin II Control of Sodium Chloride Reabsorption in the Proximal Convoluted Tubule

Consider, for example, the consequences of reduction in angiotensin II activity as shown in Table 1. The primary consequence of depression in hydrogen ion secretion in the S₁ PCT is to diminish both sodium bicarbonate and sodium chloride reabsorption in this segment. However, different load-dependent anion transport responses are observed in the S₂ PCT. For sodium bicarbonate there is excellent intrinsic ability of the S₂ PCT to compensate for the increased load emerging from the S₁ PCT (i.e., glomerulotubular balance for bicarbonate in the S₂ PCT is excellent and is little influenced by angiotensin II). Therefore, a minimal increment in sodium bicarbonate delivery out of the S₁ PCT into the loop ensues. For sodium chloride, however, the S₂ PCT cannot compensate for the augmented load leaving the S₁ PCT (i.e., glomerulotubular balance for chloride in the S₂ PCT is poor). The increment in NaCl load is therefore transmitted out of the S₂ PCT into the loop.

In summary, at constant glomerular filtration rate, an increment in angiotensin II activity that reduces S₁ PCT Na⁺-H⁺ antipporter activity results specifically in increased delivery of sodium chloride, but not of sodium bicarbonate, out of the whole PCT (Table 1). This scheme has been validated in recent experiments with saralasin and captopril in normal euvoletic rats. As discussed more fully below, a natriuresis and chloruresis occurs when angiotensin II activity is diminished because there is incomplete reabsorption by the loop and distal nephron of the increased sodium chloride load emerging from the PCT.

By physiologically regulating proximal nephron sodium chloride transport, angiotensin II should be considered an independent determinant of extracellular volume regulation. Changes in renal sodium chloride transport due directly to angiotensin II may be additive or even synergistic with changes in renal nerve activity, the peritubular Starling forces, or atrial natriuretic factor, all of which also preferentially affect sodium chloride (not sodium bicarbonate) reabsorption.

Integrative Nephron Physiology of Angiotensin II

Angiotensin II regulation of proximal sodium chloride transport does not exist in isolation, but
rather has an important impact on both the function of downstream tubular transport elements as well as on glomerular hemodynamics (via tubuloglomerular feedback). Angiotensin II also exerts significant direct effects on the renal microcirculation and, of course, on collecting duct function by regulating aldosterone biosynthesis. Thus, the interconnected, dynamic control of circulatory and tubular transport processes is clearly important in the complex governance of renal function attributable to angiotensin II.

Glomerular-Tubular Balance

Since the classic writings of Homer Smith, we know there is a coordinated linkage of the amount of solute filtered by the glomerulus and reabsorbed by the PCT. Angiotensin II, by virtue of its profound control over both glomerular and S, PCT function, is one obvious mediator of this response. Direct consequences of a change in angiotensin II activity include: modification of glomerular filtration by resetting glomerular vascular resistances (efferent>afferent) and the hydraulic permeability coefficient (Kf) and by alteration of absolute sodium chloride reabsorption in the PCT. Such parallel control of glomerular and tubular function by angiotensin II would thus directly affect the amount of sodium chloride emerging from the proximal tubule. In addition, by its presynaptic receptors and its control of filtration fraction, angiotensin II modifies two other factors that sensitively, specifically, and independently control PCT sodium chloride transport, neurogenic tone, and peritubular protein.

Sodium chloride reabsorption in the loop of Henle and distal nephron exhibits inherently good, but not flawless, load dependence. Besides intrinsically imperfect load responsiveness of distal nephron transport mechanisms, reduction in angiotensin II activity also diminishes sodium transport in the loop of Henle because of vasodilation of the medullary circulation, and in the cortical collecting tubule secondary to reduction in aldosterone level. Therefore, after reduction in angiotensin II activity, a small portion of the increment in sodium chloride and water load emerging from the proximal tubule is not reabsorbed by the distal nephron and predictably results in natriuresis, chloruresis, and diuresis.

Tubuloglomerular Feedback

Angiotensin II controls the gain by which the tubuloglomerular feedback system operates. Macula densa-mediated sodium chloride transport affects glomerular filtration, especially by regulating afferent arteriolar tone. By this indirect mechanism, angiotensin II at physiological concentrations controls the afferent arteriolar tone in addition to its direct modulation of efferent arteriolar tone. Inhibition of angiotensin II activity suppresses the tubuloglomerular feedback response. These secondary changes in glomerular filtration potentiate the impact of angiotensin II on PCT and renal solute handling.

Efferent Limb of Extracellular Volume Regulation

Dietary sodium chloride intake is sensitively tracked by reciprocal changes in renin activity and subsequently by angiotensin II and aldosterone levels. Traditionally, modulation by aldosterone of cortical collecting tubule sodium transport has been held to be important in the renal excretory response to altered dietary salt. However, a normal renal response to subtle extracellular volume changes can be observed even when aldosterone level is constant, as in an addisonian patient on fixed mineralocorticoid replacement. The data described above suggest that angiotensin II may itself participate importantly in mediating the renal response to change in dietary sodium intake or to other, more profound, perturbations in extracellular volume.

Acid-Base Disorders

When the change in angiotensin II concentration is very large, there may be physiological or pathophysiological alterations in acid-base as well as sodium chloride balance. In a primary hyperreninemic disorder, for instance, a marked deficiency in angiotensin II could lead to overtly defective Na+-H+ antiporter-mediated bicarbonate and ammonium transport in the PCT, thereby causing the metabolic acidosis usually attributed to hypoaldosteronism. Conversely, a pronounced increase in angiotensin II could enhance proximal bicarbonate absorption when filtered bicarbonate load is high. In chronic respiratory acidosis, for instance, stimulation of renin and angiotensin II by hypercapnia could be responsible for the augmented PCT bicarbonate reabsorption found in that condition.

Possible Angiotensin II–S, Proximal Convoluted Tubule Axis Dysfunction in Hypertension

By controlling 15% or more of renal sodium transport, the angiotensin II–S, cell axis is an attractive candidate for the effector site responsible for the abnormal sodium chloride reabsorption in salt-sensitive hypertensive states. Even a small change in early PCT sodium transport induced by altered angiotensin II level or cell responsiveness could quite sensitively regulate sodium chloride delivery to the distal nephron.

By predominantly affecting the earliest nephron segment, angiotensin II is in a position to control the “set point” of extracellular volume at which the kidney regulates sodium. An abnormally high blood volume, at least in relation to the level of peripheral vascular resistance, has been suggested by Guyton and others to be a primary event in some hypertensive animal models and in some patients with essential hypertension. Angiotensin II could alter the normal blood pressure–volume relation by virtue of its coordinated vascular and renal tubular actions. To overcome a primary change, S, PCT sodium reabsorption by increased local angiotensin II concentration or
response might require substantial change in extracellular volume or blood pressure.\textsuperscript{46,47} Importantly, an abnormality of the renin–angiotensin II system has been pathogenetically implicated in abnormal renal sodium transport in some forms of clinical salt-sensitive hypertension by Williams, Hol- lenberg, and others.\textsuperscript{46,49} The role of angiotensin II–S-PCT cell transport in the renal involvement for initiating or maintaining hypertension is certainly a deserving area of future investigation.

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M G Cogan

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