Mechanisms Underlying Pressure-Related Natriuresis: The Role of the Renin-Angiotensin and Prostaglandin Systems

State of the Art Lecture

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SUMMARY It has long been known that increments in renal perfusion pressure can induce an elevation of urine sodium excretion without changing renal blood flow or glomerular filtration rate. The mechanism underlying this pressure-related natriuresis remains undefined, although the interest in its elucidation has been stimulated by the notion that it may constitute the central phenomenon through which the kidney regulates blood volume and, thereby, blood pressure. Recently, the use of novel experimental techniques has disclosed some important clues about changes in renal hemodynamics that, along with changes in renal humoral regulators, allow us to visualize a possible sequence of events responsible for pressure-related natriuresis. According to this hypothesis, the autoregulatory responses responsible for maintaining glomerular filtration rate are elicited in preglomerular vasculature by changes in renal perfusion pressure. These myogenic responses are coupled through Ca2+ entry in juxtaglomerular cells with inversely related changes in the release of renin and, consequently, with the amount of angiotensin II generated in renal interstitium. The release of renin from juxtaglomerular cells is modulated by the synthesis of prostaglandin I2 from the adjacent endothelial cells. Interstitial angiotensin II could influence sodium tubular reabsorption directly by stimulating sodium transport in proximal renal tubules and indirectly by altering medullary blood flow and, thereby, medullary interstitial pressure. In the renal medulla, the effects of interstitial pressure on sodium reabsorption can be amplified by the release of prostaglandin E2 from interstitial cells. A deficient regulation of this relationship could result in a shift of the pressure-natriuresis curve, leading to hypertension. (Hypertension 11: 724–738, 1988)

KEY WORDS • prostaglandins • renin-angiotensin system • medullary blood flow • renal blood flow • glomerular filtration rate • hypertension
MECHANISMS OF PRESSURE-NATRIURESIS/ROMERO AND KNOX 725

Arterial pressure

FIGURE 1. Changes in renal plasma flow (RPF), glomerular filtration rate (GFR), and urine sodium excretion (U Na) induced by alterations in renal arterial pressure. The expression n - normal indicates that normal sodium excretion is increased by the factor n, the numbers given in the ordinate. (Reprinted from Romero JC, Fiksen-Olsen M, Schryver S. Pathophysiology of hypertension: the use of experimental models to understand the clinical features of the hypertensive disease. In: Spittel JA Jr, ed. Clinical Medicine; vol 7. Philadelphia: Harper & Row, 1981:4, with permission.)

ure-natriuresis occurs in animals undergoing water diuresis,11 a condition in which the medullary osmotic gradient has already dissipated. In spite of this demonstration, the interest in the manner in which changes in RPP could influence medullary dynamics has been revived recently by the observation made in our laboratories by Hass et al.,12 who reported in micropuncture studies that the increase of sodium excretion takes place in juxtamedullary nephrons. Furthermore, Ro-

FIGURE 2. Glomerulus with its afferent arteriole emerging from an interlobular artery and the Bowman capsule. The effer-
ent arteriole provides irrigation for the interstitial cells, which are in close contact with the tubular elements of Henle's loop and the components of the renin-angiotensin and prostaglandin systems are represented by the symbols to the right of the figure. The interrelationship among these components is explained in the text. (All = angiotensin II.)

the renal interstitium, which is responsible for adjusting the vascular tone of the efferent glomerular artery and vasa recta and for stimulating proximal tubular sodium reabsorption. These effects are represented in Figure 2 by the binding of Ang II to the efferent arteriole and to the proximal tubule.

The third relationship represented in Figure 2 is that the variations in medullary circulation induced by Ang II could alter medullary interstitial pressure, thereby stimulating the release of prostaglandin E2 (PGE2) from renal interstitial cells. Since PGE2 can decrease sodium reabsorption in the medullary portion of the thick ascending loop of Henle and in the collecting ducts, alterations in the interstitial concentration of this prostanoïd may amplify the effects of small variations of interstitial pressure on natriuresis.

In the following sections, we will review the experimental evidence that supports these concepts. Because of space limitations we have chosen to restrict the discussion to intrarenal factors. The role of other systems that integrate the renal function to general circulation, such as the atrial natriuretic factor and sympathet-

cic systems, will not be considered.

Release of Renin Viewed as a Mechanism Linked to the Myogenic Response

Bayliss94 proposed the myogenic origin of renal blood flow (RBF) autoregulation in 1902: Smooth muscle responded to changes in stretch produced by increments in blood pressure.94 This concept was later
developed to suggest that stretch should be equated to wall tension.\textsuperscript{14-15} According to the equation of Laplace, wall tension is the product of the vessel radius and the transmural pressure.\textsuperscript{20, 21} Koch\textsuperscript{21} has suggested that RBF autoregulation may result from a physical equilibrium of the arteriole, where the force tending to distend the vessel (transmural pressure) should be balanced by a force tending to constrict the vessel (wall tension). Accordingly, when the equilibrium is disturbed by increasing perfusion pressure (distending force), the vessel wall stretches and conveys a signal to stimulate the contractile elements so as to match the distending force. A new equilibrium is then reached at a higher wall tension and at a radius that is appropriate to maintain constant blood flow.\textsuperscript{20, 21}

The notion of a physical arteriole equilibrium was further developed by Frye et al.\textsuperscript{20} in a mathematical model with the introduction of a novel and attractive concept. They suggested that the signal triggered by the initial stretch of the arteriole wall can be defined by the intracellular concentration of Ca\textsuperscript{2+}. This concept is supported by strong experimental evidence. Stretching vascular smooth muscle cells increases active tension development by promoting Ca\textsuperscript{2+} entry into the cell.\textsuperscript{22} Blocking Ca\textsuperscript{2+} entry\textsuperscript{23, 24} or preventing active tension development\textsuperscript{25} abolishes autoregulation. Furthermore, contraction and relaxation of vascular smooth muscle in response to stretch is intimately associated with a Ca\textsuperscript{2+}-calmodulin-dependent phosphorylation and dephosphorylation of the 20,000-dalton myosin light chain.\textsuperscript{26, 27} Of particular interest is the finding that Ca\textsuperscript{2+} entry leading to stretch-induced contraction and phosphorylation appears to be quite separate from Ca\textsuperscript{2+} entry through activation of voltage-sensitive and hormone receptor-operated calcium channels.\textsuperscript{22, 27} This finding indicates that stretch-induced activation of Ca\textsuperscript{2+} channels may be the mechanism underlying the myogenic response of autoregulation.\textsuperscript{20} Highly relevant to the issue under discussion is the demonstration that in epithelia, stretch activates sodium entry by inserting new sodium channels from the cytoplasmic pool into the plasma membrane.\textsuperscript{28, 29} The magnitude of the active tension development can then be determined by a relationship between intracellular Ca\textsuperscript{2+} concentration and the degree of stretch that depends on the magnitude of the increase in blood pressure.

The notion of incorporating Ca\textsuperscript{2+} into the central modulatory role that links the physical disequilibrium of the arteriole to the development of active tension has other, equally important implications related to the anatomical and functional relationships of the JG apparatus. This structure is a differentiation of smooth muscle cells that have acquired the capacity to produce renin secretory granules.\textsuperscript{30} A large body of evidence has accumulated showing that the secretion of renin is inversely related to changes in intracellular Ca\textsuperscript{2+} concentration.\textsuperscript{31, 32} Every maneuver that favors an increase in Ca\textsuperscript{2+} concentrations in JG cells, such as calcium ionophores, angiotensin, norepinephrine, and arterial constriction within the range of autoregulation, decreases the release of renin.\textsuperscript{30-33} The opposite is true for the administration of Ca\textsuperscript{2+} entry blockers or during smooth muscle relaxation initiated by autoregulation.\textsuperscript{30-33} This evidence has been taken by Frye et al.\textsuperscript{20} to suggest that the localization of renin in the smooth muscle cells of afferent arterioles is also subjected to myogenic responses and that renin secretion is governed by the same conditions encompassed in the physical equilibrium of the preglomerular arterioles.\textsuperscript{30} Thus, an increase in renal arteriole stretch subsequent to an increase in RPP will integrate, through the cellular entry of Ca\textsuperscript{2+}, the vasoconstrictor response and a decrease in renin release. Conversely, a decrease in cytosolic Ca\textsuperscript{2+} triggered by a fall in wall stretch during a fall in blood pressure may account for the progressive elevation of renin release and the equally progressive vasodilatation. A mathematical model incorporating stretch as a sensor and Ca\textsuperscript{2+} as an effector for the variations in the smooth muscle tone and renin release elicited by changes in blood pressure has been recently developed.\textsuperscript{20} The equations account for the variations observed in renin release not only in response to changes in RPP but also in response to tubular and interstitial pressure elicited by ureteral obstruction. The model, however, does not contemplate any modulatory effect exerted by the release of prostaglandins on renin release.

**Involvement of the Prostaglandin System in Autoregulatory Responses and Renin Release**

It has been noted that prostaglandins modulate the release of renin. However, a better understanding of the relationship between the two systems requires the identification of the anatomical sites at which these substances are generated and the extent to which each system is compartmentalized to serve a specific function. In this section, we will focus on the specific relationship that may exist between endothelium-generated prostaglandins and the autoregulatory responses and renin release.

**Autoregulatory Responses**

Localization of synthetase activity of prostaglandins within the kidney has been performed by histochemical staining for peroxidase activity\textsuperscript{33, 34} and indirect immunofluorescence for cyclooxygenase.\textsuperscript{35} Both methods have yielded remarkably similar results. Positive immunofluorescence was observed in the endothelial cells of arteries and arterioles but not in the capillaries or venous tree.\textsuperscript{35} Glomerular epithelial cells of Bowman's capsule, the mesangium, proximal tubules, the loop of Henle, and distal convoluted tubules, including the macula densa, were negative by both immunofluorescence and peroxidase methods or showed weak and variable staining.\textsuperscript{33, 34} This result indicates that cyclooxygenase concentration in renal tissues is located predominantly in endothelial cells of preglomerular vessels.

There is a general agreement that prostaglandins are local hormones since their effects are limited to the cells where they are formed or to the neighboring cells.\textsuperscript{35, 36} With few notable exceptions, in pregnancy...
and in patients with systemic mastocytosis, the plasma concentrations of vasoactive prostaglandins are too low (≤10⁻¹¹ M) to elicit any known biological response. Moreover, there are different catabolic systems in the lung, kidney, and liver that prevent the passage of prostanooids through the circulation.

PGI₂ is a major metabolite produced in endothelial cells. The capacity of arteries to form PGI₂ is three to 10 times that of veins. It is questionable whether PGE₄, prostaglandin F₂α (PGF₂α), prostaglandin D₃, and thromboxane B₄ are formed in endothelial cells or smooth muscle in notable amounts. In renal arteries, the formation of PGI₂ elicited by acetylcholine accounts for most prostaglandin production.

The group of investigators directed by Weksler has shown that endothelial cells synthetize 10 to 20 times more PGI₂ than do smooth muscle cells. Biochemically, this difference is attributable to the fact that the concentration of prostaglandin H (PGH) synthase, and thus the capacity to form PGH₁ (the immediate endoperoxide precursor), is 20 times greater in endothelial cells than in smooth muscle. The concentration of PGI₂ synthase in endothelial cells, however, is the same as in smooth muscle.

Since PGI₂ is rapidly inactivated in the peripheral circulation, the physiological role of endothelial PGI₂ is constrained to blood elements in contact with the endothelium or to the adjacent tissues. On the luminal side of the artery, endothelial PGI₂ has been shown to regulate the activity of circulating blood cells, most notably platelets. This effect appears to be limited to the site of contact of blood cells with endothelial cells, not only because of rapid PGI₂ inactivation but because the release of PGI₂ is largely diluted in plasma. On the antiluminal (adventitial) side, PGI₂ may diffuse through the intima to act on smooth muscle layers, where the induced relaxation appears to be mediated by cyclic adenosine 3',5'-monophosphate (cAMP).

It has not been established if the amount of PGI₂ released from endothelial cells is similar from both sides of the cell or if there is an asymmetry favoring a greater flux of PGI₂ toward the antiluminal site. Studies on the subcellular localization of PGH₁ and PGI₂ synthetase and of phosphatidylinositol-specific phospholipase C and phospholipase A₂ are still inconclusive. However, as is apparent from the previous discussion, the underlying smooth muscle cells could constitute the preferential target of endothelial PGI₂. There is a wealth of information on the effects of naturally occurring principles on endothelial PGI₂. Nevertheless, the data on the effect exerted on Akt, renin release produced by a fall of RPP.

Renin Release

The relationship between PGI₂ and renin release is much better defined than the possible influence of PGI₂ on renal autoregulation. In fact, a number of investigations have demonstrated that PGI₂ is the major prostanooid responsible for modulating renin release. We have shown that in isolated glomerular preparation with afferent arteriolar attachments, renin release was elicited by PGI₂, whereas PGE₂, PGF₂α, thromboxane A₂, and endoperoxide analogues had no effect. Stimulation of renin release in the same preparations or in tissue slices by arachidonic acid was blocked by administration of specific PGI₂ synthesis blockers. Consistent with this finding are the reports of Jackson et al. showing that the constriction of the renal artery induced an increase in renal venous plasma levels of renin activity that was significantly correlated with the levels of 6-keto-prostaglandin F₁α (a major metabolite of PGI₂). Other investigators have shown that meclofenamate or indomethacin blunts the elevation of renin release produced by a fall of RPP.

From this information and from the experimental data mentioned in the previous section, one can suggest the following series of interactions among PGI₂, renin, and autoregulatory responses (see Figure 2). A
decrease of RPP induces a proportional elevation in the release of PGI₂ in endothelial cells. This increase in PGI₂ favors the relaxation of the underlying smooth muscle through an increased formation of cAMP and eicosanoids. This response will potentiate the stimulatory effects on renin release produced by a decrease in intracellular Ca²⁺ associated with the autoregulatory responses of vasodilatation.

As mentioned, the increase of renin will be followed by an elevation of Ang II in the renal interstitium, which tends to constrict postglomerular vascular segments. Ang II also tends to exert a vasoconstrictor effect on preglomerular vasculature, but such an effect will be effectively blocked by the continuous efflux of PGI₂ from the endothelial cells. In contrast, the effect of Ang II will not be counteracted in postglomerular vessels since these segments are not affected by changes in pressure and do not synthesize prostaglandins. The facilitative actions of PGI₂ on afferent arteriole vasodilatation and the constrictor effects of Ang II limited to postglomerular segments constitute two important factors involved in the preservation of GFR. Schnermann and Briggs showed that blockade of prostaglandin synthesis does not impair the maintenance of GFR at high perfusion pressure, but it significantly reduces GFR when perfusion pressure approaches the limit of autoregulation. In agreement with this observation, there are a number of studies indicating that prostaglandins may be responsible for preventing a shutdown of GFR at very low RPP.

The role of Ang II in maintaining GFR has been demonstrated by Hall.

**Segmental Resistances and Hydrostatic Pressures in Renal Vasculature**

The information available on the normal profile of hydrostatic pressure that exists along the renal vasculature in resting conditions is very incomplete. In addition, little is known about the manner in which these profiles are influenced during alterations in RPP within the range of autoregulation. However, from data given in recent reviews, we have constructed a pressure profile (Figure 3) that may exist along the renal vasculature in resting conditions as well as during changes in RPP.

**Intrarenal Profile of Vascular Hydrostatic Pressure in Resting Conditions**

In Figure 3, a mean arterial pressure of 100 mm Hg is conventionally assumed to exist during resting conditions in the arcuate artery at the level of the junction with the interlobular artery. A drop in pressure along the interlobular artery is assumed to be linear; the pressure recorded at the most distal end of the interlobular artery, near the surface of the renal cortex, is 30 mm Hg below that recorded in the aorta. A pronounced fall in blood pressure exists along the afferent and efferent arterioles. Average glomerular capillary pressures are assumed to be 45 mm Hg for superficial glomeruli and 65 mm Hg for juxtamedullary glomeruli.

The profile of the pressure in postglomerular circulation given by different investigators is very variable. In a complete review of the subject, Olstad and Aukland indicated that postglomerular resistance is markedly affected by the functional state of the kidney. On the basis of these data, we have ascribed values of 10 and 20 mm Hg at the end of superficial and juxtamedullary efferent arterioles. The existing pressure along peritubular capillaries in the renal cortex is assumed to drop from 10 to 2 mm Hg. While the drop of pressure along the vasa recta is from 20 to 1 mm Hg.

The relationship of Starling forces in peritubular capillaries, with respect to the interstitial pressures, has been extensively considered by other investigators as part of the glomerular tubular balance. These experiments have shown that the mean hydraulic pressure gradient across the peritubular capillaries is significantly lower than the peritubular capillary oncotic pressure gradient, allowing a positive net reabsorptive pressure through the end of the peritubular capillary network.

The interchange of fluid is also governed by Starling forces in the medulla, but the total amount of interchange is further complicated by the existence of the papillary osmotic gradient. A complete description of medullary interchange of fluid can be found elsewhere. We will only emphasize here that the number of microvessels in the medulla decreases from the cortical medullary junction to the papillary tip.
Effect of Changes in Renal Perfusion Pressure on the Intrarenal Profile of Vascular Hydrostatic Pressure

The expected alterations in the intrarenal vascular pressure profiles induced by changes of RPP up to 120 mm Hg or down to 80 mm Hg in the initial portions of the interlobular arteries are represented in Figure 3 by open circles and filled squares, respectively. Numerous studies have indicated that changes in RPP stimulate an active autoregulatory response in interlobular arteries and glomerular afferent arterioles but not in glomerular efferent vessels. One of the most eloquent demonstrations in this respect was provided by Gilmore et al. These investigators evoked autoregulatory responses in interlobular and afferent glomerular arterioles transplanted to the cheek pouch of the hamster by altering cheek pouch interstitial pressure. However, no responses were observed in the efferent arterioles. The study of Gilmore et al. is important because it implies that postglomerular vessels will not respond when exposed to changes in transmural pressure. This may indicate that the smooth muscle of the efferent glomerular segments are not equipped with Ca++ channels sensitive to stretch. Further studies should be performed to test the validity of this conjecture.

The profiles of intrarenal vascular pressure depicted in Figure 3 show that, because of the autoregulatory responses of preglomerular vessels, no major alterations in hydrostatic pressure are expected to occur in capillaries of superficial and deep glomeruli, efferent arterioles, and peritubular capillaries of renal cortex. In contrast, changes in hydrostatic pressure are expected to occur in juxtamedullary efferent arterioles and vasa recta in proportion to changes in RPP pressure. This concept has been derived from the studies performed by Roman et al. and Takezawa et al. These investigators have shown by the use of a laser Doppler flowmeter that changes of RPP within the range of 40 to 180 mm Hg are consistent with the interpretation given previously to illustrate the myogenic response of autoregulation. This information has been incorporated in Figure 3, showing that an increase in RPP is accompanied by a proportional increase in hydrostatic pressure at the beginning of the vasa recta to 30 mm Hg, whereas a decrease in RPP is attended by a decrease in hydrostatic pressure in the vasa recta to 10 mm Hg. As represented in Figure 3, these changes are likely to produce parallel alterations in renal interstitial pressure of ±4 mm Hg.

An important conclusion derived from these observations is that the increases in medullary interstitial pressure induced by vasodilatation of medullary vessels are likely to be transmitted to the cortex, thus affecting tubular sodium reabsorption without altering peritubular capillary pressure. This effect may explain the previously mentioned findings in microperfusion experiments, where significant decrements of sodium reabsorption in proximal tubules were observed in the absence of any alterations in peritubular capillary pressure or GFR.

Role of Intrarenal Ang II as the Humoral Mediator Responsible for Adjusting Postglomerular Hemodynamics According to Preglomerular Changes in Renal Perfusion Pressure

It has been reported that the concentrations of renin and Ang II in renal lymph effluents greatly exceed the concentrations of these substances in circulating blood. Mendelison reported that the concentration of Ang II in renal tissue exceeds, by several orders of magnitude, the levels of Ang II in blood. All the components necessary for the generation of Ang II are present in the kidney, including not only renin but renin substrate and angiotensin I (Ang I). All this evidence supports the concept that the intrarenal actions of Ang II exert important local regulatory effects.

Effects of Ang II on Efferent Arterioles and Glomerular Filtration Rate

The manner in which the intrarenal formation of Ang II could affect glomerular dynamics through alterations in afferent and efferent arteriole resistance has been the subject of intense investigation. Hall et al. have studied the effects of changes in RPP on RBF and GFR during controlled conditions and after the blockade of Ang II formation by the administration of a converting enzyme inhibitor, SQ 20881, or by an angiotensin antagonist in sodium-depleted dogs. The results of these studies, shown in Figure 5, demonstrated that after Ang II blockade, RBF autoregulation was well maintained at pressures as low as 70 mm Hg, whereas GFR autoregulation was markedly impaired. It can be seen in Figure 5 that the impairment of GFR autoregulation did not result from a major alteration in the response of preglomerular vessels (afferent arterioles), because afferent glomerular resistance fell to about the same level in the presence and absence of intrarenal angiotensin activity. The decrease in GFR autoregulation was due to the fall in efferent arteriole resistance after the blockade of Ang II. These studies are consistent with the interpretation given previously that a fall in RPP will elicit an afferent vasodilatation that reflects the myogenic response of autoregulation. Such a vasodilatation is not influenced by the progressive increase in the intrarenal concentration of Ang II, which appears to act preferentially on postglomerular vessels, thus maintaining postglomerular resistance and, thereby, GFR.

This concept is further supported by other studies indicating that, although the regulatory effects on Ang II on postglomerular vasculature are mainly exerted by intrarenally formed Ang II, circulating Ang II can act on the same site with similar efficacy. The notion of a specific effect of Ang II on postglomerular vasculature is not supported by all investigators. Some reports show that exogenous infusion of Ang II or Ang I (to stimulate Ang II formation) increases both afferent and efferent arteriole resistance. It has been...
suggested\textsuperscript{69} that these apparent discrepancies are related to an autoregulatory afferent vasoconstriction in response to an increase in systemic pressure produced by relatively large doses of Ang II. The importance of the Ang II–mediated efferent arteriole vasoconstriction is not limited only to physiological conditions; it also applies to pathophysiological conditions where this specific effect appears important for preserving glomerular function, such as in chronic renal artery stenosis\textsuperscript{92,93} and congestive heart failure.\textsuperscript{94}

We have mentioned that the release of PGI\textsubscript{2} from the endothelial cells could be the major factor in protecting afferent glomerular vessels against a progressive elevation of Ang II during a fall in RPP. In agreement with these views are the demonstrations of Olsen et al.\textsuperscript{95} showing the Ang II infusions can produce a notable afferent vasoconstriction when prostaglandin synthesis has been impaired by the previous administration of antiinflammatory drugs. The specific vasoconstrictor effect of Ang II on efferent arterioles has also been found in in vitro experiments where Ang II was applied to the adventitial side of glomerular vessels transplanted to the cheek pouch of the hamster\textsuperscript{67} or microperfused into isolated afferent and efferent arterioles.\textsuperscript{68} However, local application of large doses of Ang II may not be counteracted by the endothelial release of PGI\textsubscript{2}, particularly when performed in the absence of decrements in RPP. Under these conditions, Ang II has been shown to elicit a dose-dependent vasoconstrictor response in glomerular afferent arterioles.\textsuperscript{67}

Highly relevant to the issue under discussion are the possible effects on Ang II on the glomerular permeability coefficient ($K_g$), which has been said to be decreased by the stimulation of contractile elements contained in the mesangium.\textsuperscript{96–98} Such an effect would reduce GFR and, thereby, tubular sodium load, potentiating the sodium-retaining effect during a decrease in RPP. In fact, an Ang II receptor has been found in the glomerulus (for a review, see Reference 99), but decrements of GFR have never been documented within the range of RBF autoregulation. Quite to the contrary, the available evidence has shown the remarkable constancy of the GFR.\textsuperscript{12,79} This evidence is more in agreement with recent reports of Steinheuser et al. (see Reference 67), who could not elicit a glomerular-size reduction with a dose of Ang II capable of constricting both afferent and efferent arterioles.
Effects of Ang II on Medullary Circulation

One of the first suggestions about the possible regulatory role that Ang II could exert in renal medullary circulation was made by a group of researchers directed by S. Y. Chou. These investigators observed that a significant decrease of papillary plasma flow (PPF) was the most conspicuous alteration found during sodium-retaining states such as thoracic inferior vena cava constriction. From these findings, they suggested that the activation of the renin-angiotensin system was responsible for the decrease in PPF that was thought to induce sodium retention. To test this hypothesis, further studies were performed in which an acute sodium load was given to dogs receiving an infusion of small doses of Ang II (4 × 10^{-13} mol/kg/min) into one kidney while the other kidney was used as a control. Under these conditions, the increase in sodium excretion was significantly blunted in the Ang II-perfused kidney and unaltered in the nonperfused kidney. Increments in PPF after saline loading were significantly enhanced only in the nonperfused kidney. During all these maneuvers, GFR and RBF in both kidneys remained normal. These results were interpreted as indicating that the increase in the intrarenal level of Ang II was responsible for the decrease in PPF and, therefore, for the impaired sodium excretion. This assumption was further supported by the observation that the administration of an Ang II antagonist, saralasin, prevented the effects of Ang II on PPF and restored sodium excretion. This latter observation agrees with a recent study of Cuppies et al. showing that medullary blood flow measured by a videomicroscopic method was increased after the administration of a converting enzyme inhibitor.

The anatomical substrate for the vasoconstrictor effects of Ang II can be found in the vasa recta. The proximal portions of descending vasa recta are enveloped by smooth muscle cells that are gradually replaced by pericytes, which are particularly numerous in the inner part of the outer medulla. These pericytes contain abundant contractile filaments that are anatomically compatible with a vasomotor regulatory function. In contrast, the ascending vasa recta exhibit a thin endothelium with numerous fenestrations and contain no contractile elements.

The regulatory role of Ang II in the medullary circulation is also supported by recent studies of the distribution of Ang II receptors in the renal vasculature. Mendelsohn et al. mapped the distribution of Ang II receptors in the kidney using an in vitro autoradiographic procedure. They found a high concentration of receptors in the glomeruli spread throughout the cortex. In the medulla, a very high concentration of Ang II receptors was found in longitudinal bands that occur mainly in the inner stripe of the outer medulla. Light microscopic autoradiography confirmed that the bands of dense Ang II binding overlaid vasa recta bundles, while computerized quantitative scanning densitometry showed that the receptor density associated with the vasa recta bundles greatly exceeds that in the glomeruli. A moderately high concentration of Ang II receptors occurs throughout the inner zone of the outer medulla in the interbundle region. However, no further attempts were made to identify the anatomical structure responsible for this interbundle activity.

Control of Sodium Tubular Handling by the Intrarenal Formation of Ang II

It has long been held that the major mechanism by which Ang II alters sodium excretion is the stimulation of aldosterone secretion. However, several studies indicate that Ang II has intrarenal actions that may be even more important quantitatively than aldosterone in controlling sodium reabsorption. For example, long-term intrarenal infusion of Ang II at a rate that produces no immediate pressor effect and no increase in aldosterone secretion caused a marked sodium retention that led to increased arterial pressure within a few days of initiating the infusion.

The antinatriuretic effects of exogenous Ang II are easily demonstrable at low and moderate doses. However, at very large doses, Ang II inhibits sodium reabsorption because of the concomitant increase in blood pressure. In fact, the same high rates of Ang II infusion did not exhibit any natriuretic effect when RPP was maintained with a servocontrol device. More studies should be conducted on this matter since there are data indicating that the dual effects of Ang II on sodium transport can also be seen in microperfusion studies.

The antinatriuretic effects of Ang II can be largely explained by a direct tubular effect. In fact, with the exception of an initial microperfusion study that failed to show an effect of Ang II on proximal sodium reabsorption, subsequent micropuncture studies have demonstrated a stimulation of sodium reabsorption at doses of Ang II that range from 10^{-10} to 10^{-8} M. The blockade of Ang II production with converting enzyme inhibitors or with angiotensin antagonists also has been shown to decrease proximal reabsorption; this effect can also be observed in sympathectomized denervated kidney. More recently, Schuster et al. demonstrated a direct stimulation of net fluid reabsorption by Ang II in isolated, perfused rabbit proximal convoluted tubules. In this study, the addition of 10^{-11} M Ang II to the bath (basolateral surface) stimulated proximal tubule volume reabsorption by about 17%. Such a stimulatory effect of angiotensin was completely inhibited by the administration of 10^{-7} M saralasin. The transepithelial voltage was directly measured and found to be unchanged by Ang II, indicating that these hormonal effects are electroneutral. An important finding of all these studies is that the dose at which Ang II is capable of stimulating sodium reabsorption is lower than that obtained in renal interstitium during a decrease in RPP.

In the studies just mentioned, the micropuncture determinations were performed in superficial nephrons whereas the experiments performed on isolated proximal convoluted tubules were dissected from juxtamedullary cortex. This suggests that angiotensin can stimulate transport in proximal tubules regardless of their...
location in the cortex. Furthermore, Carmines and Navar\textsuperscript{114} have recently reported measurements of proximal tubular reabsorption in juxtamedullary nephrons in vivo. These investigators noted that the administration of converting enzyme inhibitor significantly decreased fractional proximal reabsorption, whereas the addition of 10\textsuperscript{−3} M Ang II stimulated sodium reabsorption by the proximal tubule.

The intimate mechanism by which Ang II stimulates sodium transport is not known; however, the effect appears to be receptor-mediated. Brown and Douglas\textsuperscript{115}' and Cox et al.\textsuperscript{117} have described receptors for Ang II on both brush border and basolateral membranes prepared from proximal tubules of rat and baboon kidneys. The exact role of the brush border receptors is not clear. Ang II from the luminal surface produces only a slight stimulation of sodium transport, and this response requires a dose two orders of magnitude higher than that from the basolateral side.\textsuperscript{108} On the other hand, the receptors from the basolateral membrane had an equilibrium dissociation constant of about 2 mM, appropriate for the dose response seen in intact tubules. Ang II binding is competitively displaced by saralasin.\textsuperscript{115, 117}

**Effects of Changes in Renal Medullary Flow and Sodium Excretion**

The architectural organization of the renal medulla and the manner in which vascular bundles are arranged with respect to renal tubules have been reviewed.\textsuperscript{103−105} However, we will consider some important peculiarities of the medullary interstitial cells, the possible mechanisms that can stimulate these cells to release prostaglandins, and the role of prostaglandins in the regulation of tubular sodium reabsorption.

**Medullary Interstitial Cells**

The interstitium of the inner medulla (Figure 6) contains a unique cell type, the lipid-laden interstitial cell.\textsuperscript{118} These cells are arranged in a characteristic pattern; like the rungs of a ladder, they are transversely interposed between the longitudinally running tubules and vessels. A second characteristic of the interstitial cells is the lipid droplets contained in the cytoplasm, which are composed of triglycerides and small but variable amounts of cholesterol esters and phospholipids.\textsuperscript{119} These lipid droplets do not represent storage of prostaglandins that are synthesized from arachidonic acid released from the cell membrane.\textsuperscript{118} At present, the function of these droplets is unknown.

Several theories have been advanced as to the possible functions of the interstitial cells, none of which have been appropriately validated.\textsuperscript{114} In view of the recent findings that medullary blood flow appears to be specifically altered during changes in RPP,\textsuperscript{13, 79} and that the interstitial cells are capable of releasing large amounts of PGE\textsubscript{2},\textsuperscript{93} it is tempting to postulate that these cells monitor alterations in medullary blood flow and react by producing prostaglandins that modulate reabsorption of tubular sodium. It has been shown that PGE\textsubscript{2} production from interstitial cells is favored by the entry of Ca\textsuperscript{2+}.\textsuperscript{119, 120} and that, in slices of renal medulla, the production of PGE\textsubscript{2} is dependent on the binding of intracellular Ca\textsuperscript{2+} to calmodulin.\textsuperscript{121} Stimulation of PGE\textsubscript{2} from interstitial cells has been produced by a number of naturally occurring substances,\textsuperscript{53} such as angiotensin, bradykinin, and acetylcholine, but no studies have been conducted to define the effects of medullary interstitial pressure or other physical factors related to blood flow. The demonstration that stretch could activate specific Ca\textsuperscript{2+} channels\textsuperscript{22−27} in smooth muscle cells suggests that the stretch of interstitial cells induced by an increase in blood flow or interstitial pressure could evoke the release of prostaglandins through a similar mechanism.

Deformation of interstitial cells can be produced not only by increments in interstitial pressure but also by an increase in blood volume contained in medullary vessels.\textsuperscript{8, 13, 79} This latter possibility is based on the
peculiar sheaflike structure in which vasa recta emerge from the juxtamedullary efferent arterioles ("horse tail appearance"). Such a structure provides for an initial vascular resistance arranged in parallel and individually modified downstream in direct proportion to the length of each of the vasa recta. Consequently, blood will preferentially circulate through short loop capillary networks at low levels of perfusion pressure. An increase in perfusion pressure will progressively recruit longer vessels (with higher resistance), increasing the blood flow toward the tip of the renal papilla and, thereby, the blood volume filling the renal medulla. Furthermore, this phenomenon would also imply a progressive recruitment of interstitial cells, since their number increases progressively toward the tip of the renal papilla. If the release of PGE₂ from interstitial cells is proportional to the number of cells stimulated, then the interstitial concentration of PGE₂ will increase in proportion to papillary volume filling, altering tubular sodium reabsorption in a parallel manner.

An alternative mechanism is that stimulation of interstitial cells is achieved not through a physical factor but by an increase in oxygen tension. It has been reported that an increase in oxygen tension can enhance the synthesis of PGE₂ in the renal papilla.

Release and Effects of Medullary Prostaglandins

The interstitial cells are not the only source of medullary PGE₂. PGE₂ synthesis is also present in major amounts in collecting tubules. The predominant prostanoiid synthesized in interstitial cells and collecting tubules in PGE₂, although small amounts of PGE₃ and PGI₂ have also been detected. Other elements of the renal medulla, for example, the loop of Henle, exhibit very little prostaglandin production.

To our knowledge, no experimental studies have been performed to elucidate the manner in which PGE₂ released from the renal interstitial cells acts on renal tubules. However, considering the characteristics of medullary circulation, it is conceivable that prostaglandins could pass into the ascending vasa recta and affect sodium transport in the most distal portion of proximal tubules (pars recta) or in the thick ascending loop of Henle. Such recycling routes from the ascending vasa recta to the straight tubular segment of the outer strip of the medulla have been described. PGE₂ has been shown to exert a potent blocking effect on sodium reabsorption by acting on basolateral sites of the thick ascending loop of Henle. There is no information on the effects of prostaglandins on the pars recta.

Another possible route is the diffusion of PGE₂ from interstitial cells into the ascending limb of Henle’s loop. In this case, PGE₂ could influence sodium reabsorption in thick ascending segments by acting on luminal sites. The effects of PGE₂ on thick ascending segments of Henle’s loop have not been seen by other investigators, the role of PGE₂ in sodium excretion under experimental conditions other than pressure-natriuresis remains highly controversial. In several studies PGE₂ has been shown to alter sodium reabsorption in the collecting tubule. An important characteristic recently disclosed is that PGE₂ may induce NaCl diuresis in this tubular segment not only by inhibiting NaCl reabsorption from the apical to the basolateral surface but also by enhancing chloride secretion from the basolateral to the apical surface.

An extensive study of the distribution of PGE₂ receptors in the kidney has been performed by Eriksen et al., who found that the PGE₂ receptors were located mainly in the thick ascending limb of Henle’s loop, with maximal density in the outer medullary zone. Binding to glomeruli and cortical vessels was also detected but was much less significant. These results are in accordance with the findings of Limas and Limas that the largest density of PGE₂ receptors is on the thick ascending limb of the loop of Henle collecting tubules.

Highly relevant to the issue under discussion are the studies of Pawloska et al., who determined in our laboratories the effects of renal interstitial volume expansion on fractional excretion of sodium and on the release of PGE₂ (Figure 7). Expansion of renal interstitial volume was achieved by injecting 50 μl of artificial lymph (2.5% albumin) into a chronically implanted polyethylene matrix. This maneuver produced a fractional excretion of sodium (FENa), and urine excretion of NaCl in Group 1. (Reprinted from Pawloska et al., with permission.) Asterisks indicate statistically significant changes compared with the change induced by administration of vehicle in Group 1.
4.2 ± 0.8 mm Hg increase in renal interstitial pressure, which was followed by an increment in the fractional sodium excretion of 1.02 ± 0.27% and in urinary PGE_2 excretion of 15 ± 38%. These changes were not accompanied by any significant alteration in GFR, peritubular capillary pressure, tubular pressure, or blood pressure. Inhibition of PGE_2 synthesis with meclofenamate or indomethacin prevented the increase in urinary PGE_2 excretion and fractional sodium excretion during a similar increment in intrstitial volume expansion and renal interstitial pressure. These results indicate that the release of prostaglandins could be triggered by an increase in interstitial pressure and that prostaglandins are responsible for the decrease in tubular sodium reabsorption under these experimental conditions.

Consistent with these observations are the studies of Haas et al., who showed that an increase in renal interstitial pressure results in a decrease in sodium reabsorption in juxtamedullary nephrons. These investigators have also shown that an increase in RPP from 114 ± 4 to 138 ± 5 mm Hg produced a significant decrease in the fractional reabsorption of sodium by the proximal tubule and descending limb of Henle’s loop in deep nephrons, whereas the fractional reabsorption of sodium by the superficial late proximal tubule was not changed. The results were interpreted as indicating that the decrease in sodium reabsorption during pressure is due to a mechanism that involves the renal medulla.

The demonstration of the stimulating activity exhibited by Ang II on the release of PGE_2 from interstitial cultured cells is in conflict with the scheme suggested in Figure 2. Such an effect is difficult to reconcile with the pronounced antinatriuretic action exhibited by Ang II when it is infused into the renal artery or when the intrarenal concentration of this substance is increased by renal artery constriction or a low sodium diet. However, the stimulatory effects of PGE_2 on cultured interstitial cells are not seen when Ang II is added to medullary tissue slices.

An important observation about the suggested participation of prostaglandins in mediating pressure-natriuresis was provided by Carmes et al., who showed in the salt-resistant strain of rats. These investigators showed that the blockade of prostaglandin synthesis with indomethacin reduced pressure-natriuresis by 70% without affecting GFR. They also found that there was a significant correlation between sodium excretion and urinary concentration of PGE_2 in dogs not treated with indomethacin. The same group of investigators, however, demonstrated in subsequent studies that the blunting effects of prostaglandin synthesis blockade on pressure-natriuresis were not observed when intrarenal formation of angiotensin was depressed. In fact, the administration of indomethacin to animals pretreated with a converting enzyme inhibitor failed to produce the expected reduction of pressure-natriuresis, an effect that was seen when low doses of intrarenally administered Ang II were superimposed on the converting enzyme inhibitor and indomethacin treatment. These interesting results are somewhat paradoxical because, on the one hand, they support the idea of an important modulatory role of prostaglandins on pressure-natriuresis in the presence of Ang II, while on the other hand, they deny any alteration of pressure-natriuresis in the absence of both systems. This latter idea is compatible with the existence of other mechanisms capable of regulating sodium reabsorption in the complete absence of either Ang II or prostaglandins. The validity of this proposal should await new pharmacological tools capable of inducing a complete suppression of prostaglandin synthesis without altering Ang II formation and vice versa.

Possible Involvement of Pressure-Related Natriuretic Factors in the Development of Hypertension

Guyton et al. have emphasized the central role played by pressure-natriuresis in opposing the increments in blood pressure. It is therefore important to examine the manner in which the interrelationship of physical and humoral factors, which have been mentioned in previous sections, could be disrupted so as to impair the regulation of pressure-natriuresis, thereby fostering the development of hypertension.

Impairment of Prostaglandin Release

Impairment of the ability of renal medullary interstitial cells to release PGE_2 can be singled out as a possible factor leading to hypertension. In this case, the hemodynamic signal conveyed by Ang II to the medullary circulation will not be appropriately amplified by PGE_2, resulting in a decrease in sodium excretion. This may well be the situation in Dahl salt-sensitive rats, which have a marked impairment of pressure-natriuresis as well as PGE_2 associated with a decrease both in PPF and in the synthesis of PGE_2. The hypertension in these animals is corrected on transplantsing a kidney from a salt-resistant strain of rats.

The importance of the modulatory role of prostaglandins in mediating pressure-natriuresis is highlighted by the findings of Carmes et al., who showed a blunted pressure-natriuretic effect in animals pretreated with indomethacin in which the renin-angiotensin system was assumed to remain intact. However, in this experimental design one cannot rule out the participation of a PGI_2, production of which also should have been impaired by indomethacin. According to the scheme shown in Figure 2, this latter alteration should also result in a decreased production of renin release. We have shown that, in normotensive humans, the prolonged administration of indomethacin in hypertensive (for 42 days) is followed by a persistent hyponatremia and hypoaldosteronism with hyperkalemia and marked reduction in the urinary excretion of PGE_2. The hypoaldosteronism is presumably produced by the concomitant fall in circulating levels of Ang II. These deficiencies in the renin-angiotensin-aldosterone axis were not translated in the hypotension but in a mild, although significant, increase in diastolic blood pressure. The tendency toward hypertension was presumably due to a defective sodium excretion following
altered production of prostaglandin formation in the renal medulla. In fact, these subjects exhibited reduced responses to the natriuretic effect of furosemide and impairment of the ability of the kidney to increase urine osmolality. These findings have important theoretical implications because they indicate that the repercussions of a deficient production of medullary PGE₂, which induced sodium retention, may be greater than the hypertensive effect derived from a fall in the renin-angiotensin-aldosterone axis subsequent to a deficiency of PG₁₂ production. Consistent with this thought is the mild hypertensive effects associated with hyporeninism, hypoaldosteronism, and hyperkalemia induced by indomethacin in a population of susceptible patients. A spontaneous deficiency in the synthesis of renal prostaglandins has been found in humans to be associated with hypertension, hyperkalemia, and Type IV renal tubular acidosis. The defect was corrected by the administration of furosemide. A deeper knowledge of the role of endothelial PG₁, or interstitial PGE₂, will require the development of tools that can specifically block one system without affecting the other.

Nadler et al. have described a group of patients with essential hypertension or diabetes mellitus in whom hyporeninemic hypoaldosteronism was accompanied by a reduced urinary secretion of 6-keto-prostaglandin F₁α and normal excretion of PGE₂. However, it remains unclear whether, in this group of patients, the reduction of PG₁ synthesis preceded or was a consequence of the associated disease. Muirhead et al. have described the existence of a renomedullary vasodepressor substance that is secreted from the stenotic kidney immediately after the release of renal artery constriction. This substance produces a decrease in blood pressure by decreasing total peripheral resistance. More recently, Karlström and Göthberg have indicated that vasodepressor substances can be released from nonstenotic normal kidneys when RPP is increased. If this latter observation is confirmed and the agent involved is identified as a renomedullary lipid, then one will have to regard the renal medulla as a major antihypertensive endocrine organ. In fact, the renal medulla is irrigated by vessels that can sense changes in systemic blood pressure and react by secreting agents that modulate sodium excretion and total peripheral resistance. This concept is in agreement with the development of hypertension in experimental animals after chemically induced papillectomy.

**Deficient Regulation of Intrarenal Levels of Ang II**

An impaired natriuretic response could be fostered by persistent high levels of Ang II in the renal interstitium, which may not be adequately regulated during changes in RPP. In this case, the modulation of medullary blood flow and, hence, the release of PGE₂ from interstitial cells will not be adjusted proportionately to changes of pressure in preglomerular circulation.

Such a condition has been described by Shoback et al. in a subpopulation of essential hypertensive patients in whom the levels of plasma renin activity were not decreased by either the exogenous infusion of Ang II or volume expansion. These alterations were reported to be corrected by the administration of captopril. This finding is interesting when compared with the findings of Navar et al., who reported that the administration of converting enzyme inhibitor to normotensive dogs magnified the natriuretic response induced by changes in RPP. The complexity of the interaction between physical and humoral factors involved in pressure-natriuresis makes it difficult to explain how the blockade of Ang II formation results in a much more efficient pressure-natriuresis in essential hypertensive patients and in normal animals. In the absence of basal interstitial levels of Ang II, the postglomerular vessels could remain vasodilated, allowing the alterations in RPP to be reflected in parallel changes of GFR. In this case, changes in the filtration process will add to the efficiency of the process of autoregulation, although it will render the kidney more prone to sodium losses. It is hoped that future investigations will help to explain these phenomena.

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