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Chapter 7

Renal Modulation: The Renin-Angiotensin-Aldosterone System (RAAS)

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Components of the RAAS

Ontogeny

Current Concepts and Controversies

Summary

The renin-angiotensin-aldosterone system (RAAS) plays a critical role in the maintenance of salt and water homeostasis by the kidney, particularly in hypovolemic or salt-depleted states. The net effect of the unopposed activation of this system results in sodium retention, potassium loss, and an increase in blood pressure (1).

COMPONENTS OF THE RAAS

Angiotensin Generation

Renin is synthesized in the juxtaglomerular (JG) cells (smooth muscle cells in the walls of the afferent arteriole as it enters the glomerulus), and is stored as prorenin (2–5). It is released as renin, and regulated by renal afferent arteriolar baroreceptors and the macula densa of the distal nephron of the kidney. Renin enzymatically causes the formation of angiotensin I (Ang I) from angiotensinogen. Ang I is acted upon by angiotensin converting enzyme (ACE) to form angiotensin II (Ang II). The rate limiting step in this sequence of events in humans is the release of renin, rendering it the most well regulated variable of all constituents of the Renin-Angiotensin System (RAS). Renin secretion/release is increased by three primary pathways: (i) stimulation of renal baroreceptors by a decrease in afferent arterial stretch (pressure) (6,7); (ii) stimulation of renal β_1 -adrenergic receptors, in part, through renal sympathetic nerve activity (8–10); and (iii) a decrease in sodium and chloride delivery to, and transport by, the macula densa (11,12). Renin secretion can also be regulated by several endocrine and paracrine hormones.

ACE acts on Ang I (Ang 1-10) to cleave off the active octapeptide, Ang II (Ang 1-8), which is a more potent vasoconstrictor than Ang I (13). Ang II can also be formed by non-ACE enzymes and non-renin enzymes, such as chymase, cathepsin G, cathepsin A and tissue plasminogen activator (t-PA) (14). This assumes greater significance in the organs where not all components of the RAS are expressed, providing alternate means of generation of Ang II, the physiological significance

of which is still not well understood, and may be organ specific. For example, mast cells produce renin, and have chymases, which help form Ang II and may play a role in heart failure and generation of arrhythmias (15).

ACE2 is a recently identified human homolog of ACE, sharing about 42% sequence homology. ACE2 converts Ang II to Ang 1-7. Ang 1-7 has vasodilator, antigrowth and antiproliferative properties, and exerts counterregulatory effects on Ang II. ACE 2 also decreases the level of Ang II by converting Ang I to Ang 1-9, an inactive nonapeptide (16,17). Thus, it appears that ACE and ACE2 exert opposing roles on the effects of the RAS. Unlike ACE, ACE2 is unable to inactivate bradykinin, and is insensitive to currently available ACE inhibitors. ACE2 has been identified as the receptor for the SARS-corona virus (SARS-CoV), allowing entry of the SARS-CoV into the cell (18,19). ACE2 may contribute to the localized overproduction of Ang 1-7 in the kidney, observed during pregnancy, which might protect against a rise in blood pressure (20).

Synthesis of the components of the RAAS occurs to a greater extent in certain organs relative to others, such as angiotensinogen in the liver, renin in the kidney, ACE in the lung, and aldosterone in the adrenal glands, which function together as an endocrine system. Some or all of its components are expressed in the brain, heart, vasculature, adipose tissue, pancreas, placenta, and kidney, among others, exerting autocrine, intracrine, and paracrine effects. This adds complexity to our understanding of the modulatory effects of the RAAS in maintaining homeostasis (5). Renin can be produced in the heart, brain, adrenal gland, testes, submaxillary gland, and mast cells, in addition to JG cells. Angiotensinogen is produced in extrahepatic sites such as the kidney, brain, spinal cord, mesentery, adrenal gland, atria, lung, stomach, spleen, large intestine, and ovary, and is also expressed in the ventricle and conduction tissue of the heart (5,21). ACE is ubiquitously expressed; however, the conversion of circulating Ang I to Ang II by ACE occurs mainly in the lung. ACE2 has been identified in the human heart, kidney and testis, and may be present in other tissues as well (21,22).

Ang II stimulates aldosterone synthesis and secretion by zona glomerulosa cells of the adrenal gland (23). Extra-adrenal sites of aldosterone synthesis include brain neurons, where it stimulates thirst, and cardiac myocytes, where it plays a role in the ventricular remodeling associated with salt retention (24). In these areas, the effects of aldosterone oppose those of glucocorticoids (25).

Ang II and its Metabolites

Aminopeptidases cleave Ang II at different sites. Aminopeptidase A acts on Ang II to form the heptapeptide Ang III, which participates, along with Ang II, in the classical effects on body fluid and electrolyte homeostasis, such as drinking behavior, vasopressin release and sodium appetite, in brain centers (5,21,26). Ang IV is a hexapeptide (Ang 3-8), formed by the action of aminopeptidase N on Ang II (27,28). Ang IV negatively regulates aminopeptidase A and thus influences the generation of Ang III.

Gene Targeting of Angiotensin Synthesis

The net impact of the autocrine, endocrine, intracrine, and paracrine effects of the RAAS is understood best in experimental models of controlled hyper-expression or deletion of target genes of the RAAS, such that their effects are enhanced or abolished. Most of these studies have been done in mice. Some have been performed in rats. Mice are different from other species in that they can have one or two renin genes (*Ren-1* and *Ren-2*), depending on the strain (29). *Ren-2* may have arisen from gene duplication during evolution. Thus, C57/BL6 mice express one renin gene while 129 sv mice express two renin genes. Interestingly, the adenosine receptor gene knockout mice, regardless of the strain (30), always express both

renin genes. The physiological significance of one versus two genes is unknown thus far: there is no significant difference in plasma renin levels or renal renin mRNA expression between the two.

Deficiency of individual components of the RAAS is associated with low blood pressure while over-expression of individual components of the RAAS increases blood pressure, confirming the essential role of the RAAS in maintaining blood pressure. Angiotensinogen-deficient mice exhibit profound hypotension (31,32). These mice also exhibit delayed glomerular maturation and small papillae and develop nephrosclerosis (33). Transgenic mice, which over-express the human angiotensinogen gene, develop hypertension and renal fibrosis; the latter is ameliorated by the administration of an ACE inhibitor, independent of its blood pressure lowering effects (34).

Tissue-specific targeted ablation helps to elucidate the paracrine and autocrine effects of tissue RAS. In hypertensive mice expressing human renin (hREN) and human angiotensinogen transgenes under the control of their own endogenous promoters, glial-specific deletion of angiotensinogen results in lowered blood pressure (35), indicating that the central nervous system also contributes to the regulation of blood pressure. Introduction of the mouse *Ren-2* gene into normotensive rats creates a transgenic strain that expresses *Ren-2* mRNA in the adrenal gland, and the kidney (36). This transgene provides a monogenic model for a form of sodium dependent malignant hypertension. The *Ren-2* transgenic rat has hyperproreninemia, low plasma and renal renin, high adrenal renin, and increased adrenal corticosteroid production. The hypertension that occurs in this model is responsive to ACE inhibitors. ACE deficient mice have decreased blood pressure and renal disease characterized by perivascular infiltrates and impaired concentration ability, indicating a role of ACE in the development of nephron function (37). ACE2 deficient mice develop dilated cardiomyopathy, and have a hypertensive response to Ang II (38,39).

Angiotensin Receptors

The effects of the angiotensin ligands Ang II, Ang III, and Ang IV are mediated by their interaction with specific angiotensin receptors. Ang II is known to interact mainly with two receptors, AT₁ and AT₂. Ang II and Ang III are full agonists at the AT₁ receptor, while Ang IV binds to the receptor with low affinity (27,28,33,40). Almost all the usual physiological effects of Ang II are mediated by the AT₁ receptor. The human AT₁ receptor gene is located in chromosome 3q21–q25. The human AT₂ receptor is located in chromosome Xq22–q23. AT₁ and AT₂ receptors belong to the seven-transmembrane class of G protein-coupled receptors (27,28,33,41). Adult human renal vasculature, glomeruli, and tubules (proximal, ascending limb of Henle, and collecting duct) express AT₁ receptors; AT₂ receptors are expressed in the vasculature and glomeruli but not in the tubules (42).

Rodents, but not humans, have two AT₁ receptors, AT_{1A} and AT_{1B}. They are 94% homologous, and are located in chromosome 17q12 and chromosome 2q24, respectively (43). These receptor subtypes are differentially expressed: the AT_{1A} receptor is expressed in the hypothalamus while the AT_{1B} receptor mRNA is expressed in the pituitary gland. Both AT_{1A} and AT_{1B} receptors are expressed in the adrenal gland. While aldosterone secretion is mediated by both AT_{1A} and AT_{1B} receptors in the zona glomerulosa, corticosterone secretion is mediated by AT_{1A} in the zona fasciculata (44). Adult rat kidneys express AT_{1A} receptors in blood vessels and glomeruli. More AT_{1A} receptors are expressed in collecting ducts than in other nephron segments (45,46). The AT_{1B} receptor is not expressed in the adult or fetal rat kidney (47). In adult rat kidneys, AT₂ receptors are expressed to a greater extent in glomeruli and to a lesser extent in proximal tubules and blood vessels. Female rats express more AT₂ receptors than male rats (48).

Occupation of the AT₁ receptor by Ang II triggers the generation of various second messengers via heteromeric G-proteins, chiefly G_{q/11}. Phospholipase C (PLC) β1 is activated leading to the formation of 1,4,5-inositol trisphosphate (IP₃) and diacylglycerol (DAG) from the hydrolysis of phosphatidylinositol-4,5-bisphosphonate (PIP₂). IP₃ activates IP₃ receptors in the endoplasmic reticulum releasing Ca²⁺. Ca²⁺ released from the endoplasmic reticulum causes the Ca²⁺-sensing STIM1 protein to interact with Orai1 in the plasma membrane. This interaction and the activation of IP₃ receptors at the plasma membrane allow the influx of extracellular calcium (49,50). The increase in intracellular calcium and the stimulation of protein kinase C (PKC) by DAG lead to vasoconstriction (51). Activation of the AT₁ receptor stimulates growth factor pathways such as tyrosine phosphorylation and PLCγ activation, leading to activation of downstream proteins, including mitogen-activated protein (MAP) kinases, signal transducers and activators of transcription (STAT protein). These cellular proliferative pathways, mediated by the AT₁ receptor, have been implicated in the proliferative changes seen in cardiovascular and renal diseases (52).

The AT₁ and AT₂ receptors are differentiated based on their affinity for various non-peptide antagonists (51,53). The AT₂ receptor shares 32–34% amino acid homology with the AT₁ receptor, but activates second messenger systems with opposite effects via various signal transduction systems, mainly G_i and G_o proteins (27,28,33,41,49,54). Stimulation of the AT₂ receptor leads to activation of various phosphatases, resulting in inactivation of extracellular signal-regulated kinase (ERK), opening of K⁺ channels and inhibition of T-type Ca²⁺ channels. The AT₂ receptor has a higher affinity for Ang III than Ang II. AT₂ receptors may mediate antiproliferative effects, apoptosis, differentiation, and possibly vasodilatation.

Gene Targeting of Angiotensin Receptors

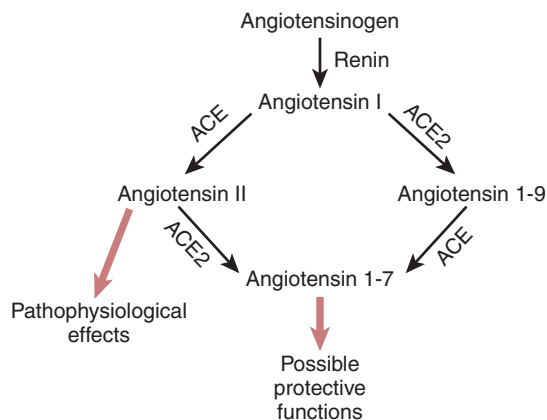
Gene targeting experiments in mice have elucidated the effects of angiotensin receptors. AT_{1A} knockout mice are hypotensive and have a mild reduction in survival, compared to angiotensinogen knockout mice (41,55,56). AT_{1A} receptor knockout mice have minor anomalies in the renal inner medulla and papillary structure (41,55–59). AT_{1B} receptor knockout mice show no specific abnormalities and have normal blood pressure. This indicates that the AT_{1A} receptor can compensate for the absence of the AT_{1B} receptor (60). However, mice lacking both AT_{1A} and AT_{1B} receptors show deficient urinary peristalsis during the perinatal period (61). AT₂ receptor knockout mice have modest elevation of blood pressure, and an enhanced pressor response to Ang II (62–64).

Several proteins interact with the AT₁ and AT₂ receptors. Over-expression of the AT₁ receptor-associated protein (ATRAP) decreases AT₁ receptor number (65). In contrast, the AT₂ receptor-interacting protein (ATIP1) cooperates with the AT₂ receptor in the inactivation of receptor tyrosine kinases, thus playing a role in the initiation of a signaling cascade that inhibits cell growth (66).

Other Ang II Receptors

There are two other receptors for Ang II. The AT₃ receptor represents an angiotensin binding site and is identified in a mouse neuroblastoma cell line (67). The AT₃ receptor has a high affinity for Ang II but a low affinity for Ang III. The AT₄ receptor is an angiotensin binding site, with a high affinity for Ang IV (27,40,68). Unlike the AT₁ and AT₂ receptors, the AT₄ receptor is not coupled to heterotrimeric G proteins, and has been identified as an insulin-regulated transmembrane aminopeptidase (IRAP). A unique binding site for the heptapeptide Ang 1-7 (formed by the action of ACE2 on Ang I) has also been identified (Fig. 7-1).

Figure 7-1 Pathways of ANG generation. (After Danilczyk U, Penninger M: Angiotensin-converting enzyme II in the heart and kidney. *Circ Res*, 98(4): 463–471, 2006.) ACE2-regulated pathways: both ACE and ACE2 are involved in the production of the biologically active peptides Ang II and Ang1-7 from Ang I. Elevated levels of Ang II are known to be detrimental to the function of the heart and kidney. Ang1-7 may function as a key peptide involved in cardioprotection and renoprotection. Genetic experiments suggest that ACE and ACE2 have complementary functions by negatively regulating different RAS products. The fine details of their regulatory function may differ depending on the local RAS environment.



Physiological Effects of Ang II

Ang II exerts most of its physiological effects via the AT_1 receptor. Ang II has pleiotropic actions (69), including direct and indirect vasopressor effects. In response to sodium depletion, hypotension and/or hypovolemia, Ang II is formed, which causes immediate vasoconstriction of arteries and veins, increasing peripheral vascular resistance and venous return respectively, and raising blood pressure. The effect of Ang II on blood pressure secondary to increased ion transport by the renal tubule is more gradual. Ang II increases sodium and chloride reabsorption directly in several segments of the nephron. In the proximal tubule, low concentrations of Ang II play a central role in ion transport by activating luminal Na^+/H^+ exchanger (NHE), Na-glucose cotransporter (70), sodium phosphate cotransporter (NaPi) (71), basolateral $Na^+K^+ATPase$ (72,73) and $Na^+-HCO_3^-$ exchanger (74). AT_1 receptors stimulate NHE3 (75–77) but not NHE2 (78). High concentrations of Ang II can inhibit proximal tubule sodium transport via the stimulation of PLA₂- and cytochrome P-450-dependent metabolites of arachidonic acid (74,79–82). Ang II also affects ion transport in the medullary thick ascending limb of the loop of Henle in a biphasic manner (83). In this nephron segment, low concentrations of Ang II increase sodium, potassium, and chloride transport by stimulating NHE3 and NaK2Cl cotransporter activities (84). Ang II stimulates NHE1 activity in the macula densa (85). Ang II also stimulates NHE2 in the distal convoluted tubule and amiloride-sensitive Na^+ transport (epithelial sodium channel, ENaC) in the collecting duct (86). All these effects are mediated by the AT_1 receptors.

The role of AT_2 receptors in influencing sodium transport is not well established. AT_2 receptors may inhibit sodium transport. AT_2 receptors are coupled to NHE6 (87), but NHE6 is not involved in sodium reabsorption (88). Ang 1-7 has been reported to inhibit $Na^+K^+ATPase$ in pig outer cortical nephrons (89), and $Na^+-HCO_3^-$ exchanger in mouse renal proximal tubules (90). However, an increase in sodium transport via AT_2 receptors in rat proximal tubules has also been reported (91). These discrepancies may be related to the condition of the animal. For example, AT_2 receptors inhibit $Na^+K^+ATPase$ activity in renal proximal tubules of obese but not lean rats (92), and during AT_1 receptor blockade (93).

Ang II indirectly increases sodium transport by stimulating the synthesis of aldosterone in the zona glomerulosa of the adrenal cortex. Aldosterone increases the density of ENaC in the cortical collecting duct by inducing the transcription of α -ENaC and redistribution of α -ENaC in the early cortical collecting duct from a cytoplasmic to an apical location. However, Ang II can stimulate the expression of α , β , and γ ENaC, independent of aldosterone (94). Aldosterone also activates

$\text{Na}^+\text{K}^+\text{ATPase}$ in the basolateral membrane of the principal cells of the cortical collecting duct. Aldosterone, like Ang II, can act in an autocrine and paracrine manner. Aldosterone has been reported to be produced by aldosterone producing cells other than the adrenal glomerulosa, such as neuronal glial cells and cardiac myocytes (95).

There is evidence that Ang I and Ang II may act, in ligand-independent ways, to affect cell signaling, cell–cell communication and growth, via their metabolism by the enzyme ACE2, mentioned above. They may also oppose the traditionally accepted effects of the RAAS (96). While AT_1 receptors can be stimulated by stretch, independent of Ang II (97), Ang II can have intracellular effects independent of AT_1 receptors (98).

Concepts and Controversies in our Current Understanding of the Renal Effects of the RAAS in Maintaining Fluid and Electrolyte Homeostasis and Blood Pressure

Feedback mechanisms in the kidney contribute to the maintenance of renal blood flow and glomerular filtration rate (GFR) in the face of fluctuations in blood pressure. This phenomenon of renal autoregulation was first recognized as early as 1932 (99). In some animals, autoregulation of renal blood flow is negligible at birth (100). In primates, in the immediate neonatal period, autoregulation of renal blood flow is present, but autoregulation of blood flow to other organs, such as

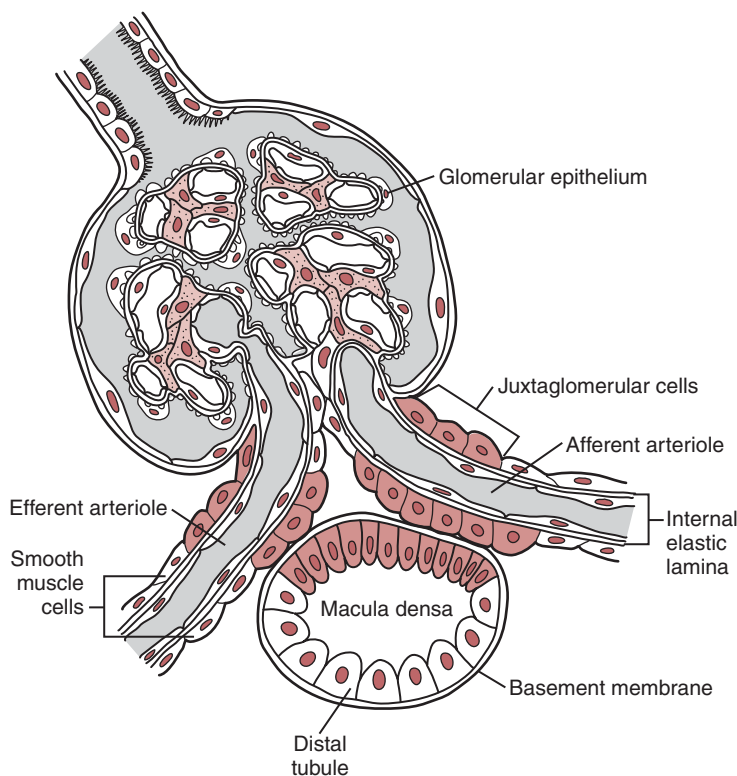


Figure 7-2 The juxtaglomerular apparatus and tubuloglomerular feedback (TGF). (From Guyton AC, Hall JE: The kidneys and body fluids. In Guyton AC, Hall JE (eds): Textbook of Medical Physiology (Fig 26.14, p. 292). Philadelphia, PA: WB Saunders, 2000.) TGF is achieved because of the anatomy of the nephron and juxtaglomerular (JG) apparatus. TGF is a phenomenon that occurs when changes in tubular fluid concentrations of Na^+ , Cl^- , and K^+ are sensed by the macula densa, via the luminal $\text{Na}^+\text{-K}^+\text{-2Cl}^-$ cotransporter. Increases or decreases in luminal uptake of these ions cause reciprocal changes in GFR by alterations in vascular tone, mainly in the afferent arteriole.

brain, myocardium, and intestines, is not observed (101). Autoregulation of renal blood flow is observed in canine puppies as young as 14 days of age (102). However the set point is lower and the efficiency of autoregulation is less in younger than in older puppies.

Autoregulation is achieved by the interplay of two mechanisms: (i) tubuloglomerular feedback (TGF), which involves a flow-dependent signal from the macula densa and alteration of afferent arteriolar tone, which is mediated by adenosine and/or ATP (103–105); and (ii) myogenic response, which involves direct vasoconstriction of the afferent arteriole in response to increased transmural pressure. These two mechanisms act in concert to prevent acute fluctuations in renal blood flow and GFR in response to changes in blood pressure (Fig. 7-2) (106).

Recent studies challenge pre-existing concepts and merit discussion. Glomerulotubular balance (GTB) is a negative feedback loop that occurs when proximal tubular reabsorption of sodium and water increases in response to increased GFR (107), and vice versa. Thus, GTB affects distal sodium delivery and, consequently, TGF. TGF and GTB may be more critical in the regulation of renal function in the neonate than in the adult as sodium intake is low in the newborn; sodium balance must be tightly regulated to achieve the net positive balance required for growth. Recent advances in our understanding of GTB, specifically in the neonate, will be discussed.

ONTOGENY

Development of the RAAS: Structure of the Kidney and Urinary Tract

Prenatal Changes in RAAS Structure and Function in Rodents and Humans

STUDIES IN HUMANS

In humans, angiotensin-related genes are activated at Stage 11 of the developing embryo (108), which corresponds to 23–24 days of gestation. AT₁ and AT₂ receptors are expressed very early (24 days of gestation), indicating that Ang II may play a role in organogenesis. The AT₁ receptor is expressed in the glomeruli while the AT₂ receptor is found in the undifferentiated mesonephros that surrounds the primitive tubules and glomeruli. AT₂ receptor is maximally expressed at about 8 weeks of gestation, followed by decreasing, but persistent expression until about 20 weeks of gestation (109). At Stage 12–13, which corresponds to 25–29 days of gestation, angiotensinogen is expressed in the proximal part of the primitive tubule, while renin is expressed in glomeruli and JG arterioles. ACE is detected in the mesonephric tubules at stage 14. By 30–35 days, all components of the RAAS are expressed in the human embryonic mesonephros. Expression of these proteins in the future collecting system occurs later, at about 8 weeks of gestation.

ACE has a role in fetal growth and development. ACE inhibitor-induced fetopathy, consisting of oligohydramnios, intrauterine growth retardation, hypocalvaria, renal dysplasia, anuria, and death, has been described in mothers exposed to ACE inhibitors during the second and third trimesters of pregnancy (110). These effects were initially believed to arise from a decrease in organ perfusion (111). More recent evidence indicates that ACE inhibition has teratogenic effects during development. Fetuses exposed to ACE inhibitors during the first trimester have an increased risk of congenital malformations, with an incidence of 7.1%, compared to those with no maternal exposure to antihypertensive medications during the same time period. The congenital anomalies are major and include cardiovascular defects such as atrial septal defect, patent ductus arteriosus, ventricular septal defects and pulmonic stenosis; skeletal malformations, including polydactyly, upper limb defects and craniofacial

anomalies; gastrointestinal malformations such as pyloric stenosis and intestinal atresia; central nervous system malformations such as hydrocephalus, spina bifida and microcephaly; and genitourinary malformations including renal malformations (112). Angiotensin receptor blockers (ARB) have also been reported to be fetotoxic (113). Inhibition of the activity of the RAAS in pregnancy may have effects on the fetus that manifest later on in life, such as hypertension, which are addressed later.

STUDIES IN RODENTS

The expression patterns of the RAS components in the embryonic kidney are similar in rodents and humans (108,114). Angiotensinogen is expressed in the proximal part of the primitive tubule in the mouse at embryonic day 12.5 (30 days' gestation in humans) (115). Renin is widely expressed initially in the renal vasculature in the embryonic rat, but its expression becomes localized to the afferent arteriole with development. Renin secretion is greater during the early embryonic period and decreases gradually. Circulating renin levels are higher in the neonatal rodent than in the adult, and appear to be due to increased production (115). Rodent ACE follows a similar expression pattern as in humans. Ang II and AT₁ receptors are important in nephron development and afferent arterial growth. AT₁ receptors may guide Ang II to its target sites to induce cell growth and differentiation (116–118). AT₁ receptor mRNA is strongly expressed during the early embryonic period (E17 to E20 in the rat), with increased expression in mature nephron segments by E17, persisting until adulthood (119). AT₂ expression is seen in both the undifferentiated mesenchyme in the maturing kidney and the mature nephron, including the proximal tubule. Collectrin, a novel homolog of ACE2 (120) shares about 47% homology with ACE2 in humans, but lacks the carboxypeptidase function. Collectrin mRNA is expressed in the mouse embryo at day 13, is localized to the ureteric bud, the progenitor of the collecting duct, increases throughout development, and decreases after birth.

Mutations of genes encoding renin, angiotensinogen, ACE, or AT₁ receptor are associated with skull ossification defects (121), as well as autosomal recessive renal tubular dysgenesis (122–124). AT₁ receptor deficient mice do not develop a renal pelvis (122). AT₂ receptor deficient mice have congenital abnormalities of the kidney and urinary tract (CAKUT), such as multicystic dysplastic, and aplastic kidneys (124). The more dramatic phenotypes seen in mice with deficient AT₂ receptor, as compared to humans with similar defects, is attributed to the fact that human nephrogenesis is completed in utero, while maturation of rodent nephrons occurs for up to 10 days after birth. However, AT₁ receptor gene deficient phenotypes are similar in rodents and humans. As indicated earlier, mutations in angiotensin-related genes in humans are associated with renal tubular dysgenesis (123). These studies demonstrate the importance of the RAAS in development and maturation of the kidney and collecting systems (115).

Postnatal Changes in RAAS Structure and Function in Rodents and Humans

STUDIES IN RODENTS

In rats, angiotensinogen mRNA expression is negligible immediately after birth, but increases dramatically by day 5, only to decrease again over the next few days, followed by a gradual increase to adult levels by 2 months of life. The newborn rat kidney can generate Ang II directly from angiotensinogen via a serine-protease that is induced in the newborn (125). Renin levels are higher in the neonatal than the adult rodent, and appear to be due to increased production (107). Ang II levels are high in the early neonatal period, and decrease with age.

STUDIES IN HUMANS

The RAAS is more highly activated in the neonatal period and infancy compared to later in childhood (126). Plasma renin activity and plasma aldosterone levels are markedly increased in preterm human infants in the first 3 weeks of life (127). While fetal ACE levels remain stable during gestation in rats (128), serum ACE activity has been reported by some to be higher in late fetal than in neonatal life in humans (128–130), lambs and guinea pigs (131,132). In contrast, renal ACE activity may increase with age, at least in pigs and horses (133,134). A similar pattern may be found in humans based on measurement of urine ACE isoforms (135). Recent studies measuring plasma Ang II levels in 46 normal and low birth weight human infants have shown increased Ang II levels at 7 days of life in very low birth weight infants (136). Maximum serum aldosterone levels are seen at 2 h of age, and gradually decrease over the first year of life (137).

Sodium Homeostasis in the Neonatal Period

Sodium intake is low in infants compared to adults, as milk is a poor source of sodium. However, a positive balance of sodium in the neonate is needed to sustain growth, in contrast to normal adults who are in sodium balance. The kidney of a full term infant filters 4–5% of the volume of plasma filtered by an adult (120 mL/min/1.73M²). Despite the relative paucity of sodium transporters, the full-term neonate does retain sodium, in part because of the low GFR (3). Although renal sodium transporters are reduced, neonates cannot excrete a sodium load compared to adults. This has been attributed to the increased activity of agents that increase sodium reabsorption in the neonatal period, including the RAAS and α -adrenergic receptors (117,138). Endogenous Ang II inhibits the natriuresis of acute expansion in the neonatal rat (139). The decreased effects of natriuretic factors (e.g., atrial natriuretic peptide, dopamine, nitric oxide) in the term neonate compared to the adult may also impair its ability to excrete a sodium load (140–142). Water transport across the proximal tubule also differs in adults and neonates. The adult tubule transports water by the water channels called aquaporins, which belong to the category of non-solute carrier-related genes. These are present in very low concentrations in the neonatal nephron. However, water is transported effectively in the neonatal nephron by paracellular and transmembrane mechanisms and by passage through non-aquaporin channels to maintain GTB (see below) (143). Aquaporin expression increased with age, an effect that is mediated by glucocorticoids (144).

Development of TGF

Growth and maturation affect the influence of distal ion delivery on the sensors of the macula densa in producing a vascular response. TGF responses are operative in neonatal rats, as early as 3.5 weeks of age (145,146). The inflection point for TGF may also be different between younger and older rats. Extracellular fluid volume and dietary protein affect the TGF response profoundly. The effect of dietary protein on the TGF response varies in different studies, with high protein diets stimulating or blunting the TGF response.

Despite a lower GFR, urinary sodium losses are highest in the most premature babies but fractional sodium excretion (FE_{Na}) is exponentially related to gestational age. The renal sodium loss in prematurity appears, in part, to result from the immaturity of the TGF mechanism. Postnatal age has been shown to have an independent effect on FE_{Na} but not on GFR. These findings indicate that in infants of greater than 33 weeks' gestation, sodium conservation is possible because of adequate TGF. The rapid increase in sodium reabsorption in the first few postnatal days seems to be due to maturation of distal tubular function. Although this was

initially attributed to aldosterone (147), the decreased amount of transporters that are targets of aldosterone makes this mechanism unlikely. An increase in maturity of the $\text{Na}^+\text{K}^+\text{ATPase}$ in the distal tubule and a decrease in extracellular fluid volume may be contributory factors to postnatal maturation of sodium transport in the distal tubule.

Development of GTB

The essential regulatory mechanisms of tubule transport include GTB and neural and hormonal factors such as sympathetic innervation, Ang II, endothelin, parathormone, and other mediators. As defined earlier, GTB is the capacity of each segment of the nephron to reabsorb a constant fraction of the GFR, fluid and solute delivered to it, and is influenced by peritubular and intratubular capillary pressures and GFR (148). The capacity of the proximal tubule to reabsorb sodium, chloride and water, bicarbonate, glucose and organic substances is adjusted to GFR: the higher the GFR, the greater the reabsorption. The underlying physical mechanism of GTB is a flow-dependent reabsorption of ions and water across the renal proximal tubular luminal membrane in response to changes in GFR, which is independent of neural and hormonal systems. Maintenance of GTB is influenced by flow rate, substrate delivery and other unknown systems. It is signaled by the hydrodynamic torque (bending movement) on epithelial microvilli (149). Increases in luminal diameter have the effect of blunting the impact of flow velocity on microvillous shear stress and, thus, on microvillous torque. Variations in microvillous torque produce nearly identical fractional changes in sodium reabsorption. Furthermore, the flow-dependent sodium transport is enhanced by increasing luminal fluid viscosity, diminished in NHE3 knockout mice, and abolished by non-toxic disruption of the actin cytoskeleton. These data suggest that the 'brush-border' microvilli serve a mechanosensory function wherein fluid dynamic torque is transmitted to the actin cytoskeleton to modulate sodium absorption in renal proximal tubules.

Clinical studies have been conducted to study GTB in term and preterm human infants, which suggest that GTB is operational from about 33 weeks of gestational age (147). In a study of 70 infants of gestational ages 27–40 weeks and postnatal ages 3–68 days, 24-h sodium balance studies and creatinine clearance measurements showed that intrauterine age and extrauterine existence independently increased the maturation of this function. The incidence of hyponatremia was associated with a negative sodium balance, which varied directly with the degree of prematurity. Indeed, no babies born after 36 weeks had a negative sodium balance. Studies in rats also show that GTB is present in 4.5 month old rats. Thus, it appears that GTB is maintained during development by proportional maturation of GFR and tubular reabsorptive mechanisms (150).

CURRENT CONCEPTS AND CONTROVERSIES

The changes in preglomerular resistance that regulate renal blood flow and GFR in the face of changing blood pressure is attributed to TGF and the renal myogenic response, acting in concert, as mentioned earlier. This is the classic paradigm. However, over the last 20 years, the importance of TGF and renal autoregulation has been challenged, and the role of the myogenic response as a renoprotective mechanism to prevent renal damage due to increased blood pressure has been demonstrated in several studies.

There is evidence that renal protection is lost when autoregulation fails (151,152). The changes in glomerular capillary pressure to maintain GFR at a relatively constant level in the face of increasing or decreasing blood pressure is

different from the vascular response needed to reduce GFR to limit pressure-induced increases, which cause renal injury. Thus, TGF and the myogenic response occur at different levels and may have different goals in the balance between renal autoregulation and renal protection. Classic studies in the two kidney, one clip model of hypertension (153,154) were followed by studies in the uninephrectomized deoxycorticosterone acetate (DOCA)/salt model of malignant nephrosclerosis (155), confirming the damage engendered by a dilated renal vascular bed in the face of hypertension. In a 5/6 ablation model of chronic kidney disease, the loss of autoregulation increases susceptibility to hypertensive renal injury.

There are different requirements for protection versus regulation. The myogenic response, which constricts the afferent arteriole, occurs after every 3–4 s in response to a blood pressure change. A TGF response takes up to 20 s, as it involves the sequence of increased distal delivery, sensing by the macula densa, release of mediator (now presumed to be adenosine/ATP) and generation of arteriolar afferent response (156). Thus, very brief perturbations of blood pressure have insignificant effects on renal blood flow and GFR. Such brief episodes should alter the myogenic response, but there are no convincing *in vivo* data available that support this. Similarly, there are no studies on the effects of spikes in systolic versus spikes in mean arterial blood pressure in generating a renal myogenic response. A delay in the onset of pressure-induced vasoconstriction has been reported in the intact kidney (157), with a longer delay in vasodilatation induced by decreasing blood pressure in the intact kidney and an even longer delay in a hydronephrotic kidney.

The advocates for TGF as the dominant mechanism argue that as GFR is influenced by several factors, including plasma colloid pressure, proximal tubular pressure and the filtration coefficient (158), a vascular response to changes in pressure alone may not be adequate for regulation. TGF, with its response to alteration in distal sodium delivery, may thus play the stronger autoregulatory role, while its immediate renoprotective role may be less important.

Differentiating these two closely aligned mechanisms is difficult. In the Fawn-Hooded rat, the Brown-Norway rat, and the Dahl salt-sensitive rat, the genetic defect in autoregulation seems to involve the myogenic response, with an intact or even enhanced TGF (159–161). Studies of renal injury in these models may shed more light on this issue. We do know that humans with uncomplicated, essential hypertension and intact renal autoregulation do not exhibit renal injury (151,152).

If autoregulation is essential for volume homeostasis, one would expect an unequivocal relationship between the two. However, there is little evidence that impaired autoregulation leads to impaired volume homeostasis. Hypertension is not clearly linked to loss of autoregulation. In the Brown-Norway rat, blood pressure is reduced and administration of DOCA or NaCl has little effect (162). In genetic knockout mice without the TGF mechanism, no overt volume disturbances are noted (163). Similarly, if distal delivery is manipulated, such as by the chronic use of loop diuretics, the effects should be catastrophic, since these also suppress TGF. However after an initial loss of volume, steady state adaptations occur within 3–4 days. GTB helps in this adjustment.

Mediators and Modulators of TGF

There are several recent studies aimed at identifying the mediator of the TGF mechanism, which is critical to autoregulation. As defined earlier, TGF is a phenomenon that occurs when changes in tubular ion transport by the macula densa cause reciprocal changes in GFR by alterations in vascular tone, mainly in the afferent arteriole (106). The initial step in the TGF mechanism is the sensing of the luminal signal by the $\text{Na}^+\text{K}^+\text{2Cl}$ cotransporter at the macula densa. The activation of the $\text{Na}^+\text{K}^+\text{2Cl}$ cotransporter generates adenosine, which stimulates A_1

adenosine (A_1) receptors, resulting in increased cytosolic calcium. Some investigators have suggested that ATP, activating P2X1 receptors, triggers the increase in cytosolic calcium. The calcium signal is propagated to extraglomerular mesangial cells, constricting vascular smooth muscle cells of the afferent arteriole and decreasing GFR. Renin secretion is also inhibited, which allows recovery of arteriolar flow and GFR. TGF is absent in A_1 receptor knockout mice. In contrast, TGF response persists in mice in which the ACE, AT_1 receptor, NOS1, or thromboxane receptor genes are disrupted (164). Vasoconstrictors, such as Ang II, increase the sensitivity of the TGF response, while vasodilators, such as nitric oxide, blunt the response (106). These studies indicate that TGF is modulated by Ang II, arachidonic acid metabolites, and nitric oxide, while the primary mediators are adenosine and/or ATP.

Adenosine

A recent study of $cd73^{-/-}$ mice, which cannot generate ATP/adenosine, suggests that a humoral factor, adenosine, may mediate TGF response. Thus, a lack of adenosine abrogates the change in GFR engendered by TGF, but does not affect distal reabsorption of sodium and water along the tubule, likely mediated by aldosterone (165). The cells of the macula densa display a role in regulation, by sensing increased NaCl delivery to the distal tubule, and activation of Na^+K^+2Cl cotransporter activity to reduce GFR, with adenosine most likely being the mediator of this response. Indeed, TGF is absent in A_1 receptor knockout mice (165). In anesthetized wild type, and A_1 receptor knockout mice, GFR and renal blood flow were measured before and after reducing renal perfusion pressure by a suprarenal aortic clamp. A reduction in blood pressure produced a significantly greater fall in A_1 receptor knockout mice compared to the wild type, indicating reduced regulatory responses in the knockout mice. This suggests that deficient autoregulation in the absence of the effector adenosine or A_1 receptor is mediated by abrogation of the TGF response.

The administration of highly selective adenosine 1 receptor antagonist, CVT-124, results in marked diuresis and natriuresis, indicating failure of autoregulation (148). The diuresis and natriuresis are associated with a reduction in absolute proximal tubular reabsorption and its uncoupling from glomerular filtration. In addition, the response of the macula densa to increased distal delivery of sodium chloride and water is blunted, as there is no corresponding decrease in GFR. This indicates inhibition of the TGF mechanisms, and provides additional support for adenosine being the mediator of the TGF response. The lack of proximal tubular regulation in GTB could be indicative of adenosine being a player in GTB, in addition to the known and accepted peritubular, luminal and oncotic controls that regulate it. However, the mechanism underlying the increased formation of adenosine in response to an increase in Na^+K^+2Cl cotransporter activity remains to be determined.

ATP

Navar et al (166) make the case for ATP being the mediator of the TGF response based on their finding that ATP selectively affects renal afferent arteriolar tone, the demonstration of the presence of ATP-specific receptors P2X1 in the renal afferent arteriole, the absence of the TGF response in mice lacking the P2X1 receptor, and evidence that ATP directly stimulates the L-type voltage gated calcium channel, leading to calcium influx and vascular smooth muscle cell contraction (167,168).

Nitric Oxide (NO)

NO, derived from arginine, modulates TGF. Type I NO synthase (NOS) is expressed in the macula densa (169). Other NOS isoforms may be expressed in the mesangium and glomerular microvessels. These enzymes are strategically positioned to

influence each step of the TGF process (170). However, micropuncture experiments using NOS antagonists have shown that NO does not mediate TGF. Instead, local NOS blockade causes the curve that represents TGF to shift leftward and become steeper. Changes in NO production in the macula densa may underlie the resetting of TGF, which is needed to maintain the TGF curve so that it adapts to different conditions of ambient tubular flow to accommodate physiologic circumstances and maintain homeostasis (171). Also, macula densa NO production may be substrate limited and dissociated from NOS protein content. The importance of NO to TGF resetting and the substrate dependence of NO production have both been found during changes in dietary salt. Changes in neuronal NOS (nNOS or NOS1) function have been shown to occur in the JGA of the spontaneously hypertensive rat (SHR) (172). NOS inhibition has been demonstrated to have a reduced effect on TGF in the SHR (173).

Reactive Oxygen Species

Reactive oxygen species and ongoing oxidative stress are increasingly implicated in the pathophysiology of vascular changes in many diseases, including hypertension. AT_1 receptors increase the sensitivity of the TGF response, which could also be related to reactive oxygen species. While the superoxide anion may lead to vasoconstriction and an increase in myogenic tone, directly, and via many signaling pathways, it also influences vasoconstriction and vasodilatation as a consequence of TGF. These effects occur due to changes in macula densa cell function in response to changes in sodium chloride delivery to the distal tubule. The macula densa expresses nNOS (171), which is activated during sodium chloride reabsorption and has a vasodilatory effect; this blunts the vasoconstriction caused by the TGF in response to increased sodium chloride transport. Oxygen radicals enhance the TGF response and limit NO signaling from the macula densa (174). Reactive oxygen species react with NO, producing peroxynitrate, which impairs vasorelaxation and promotes hypertrophy (175–177): thus reactive oxygen species effectively counteract the effects of NO.

The interaction between NO and superoxide anion has been studied in microperfusion experiments. The infusion of a nitric oxide precursor into the macula densa caused a graded reduction in the TGF-mediated afferent arteriolar vasoconstriction; this response was more pronounced in the Wistar Kyoto (WKY) rat compared to the (SHR) (171). The thick ascending limb of the loop of Henle also produces NO, as it expresses endothelial NOS (eNOS or NOS III) (170,173). Here, studies have shown that NO decreases net absorption of chloride and bicarbonate in the isolated thick ascending limb (178), thus indirectly decreasing the TGF response by increasing delivery of these to the distal tubule. Superoxide anion activates 5'-nucleotidase, thereby increasing adenosine generation in the kidney (which we know to be a mediator of TGF). These studies implicate reactive oxygen species in the enhancement of TGF (106).

Calcium Wave

Intracellular calcium modulates vascular smooth muscle tone and is the mediator for stimulation of renin release. There is evidence for a calcium wave that spreads through the mesangial cell field and constricts the afferent arteriolar smooth muscle cells. It appears that both gap junctional communication and extracellular ATP are integral components of the TGF calcium wave. The finding that the calcium wave is generated by ATP, but not by adenosine, offers a new model for a direct effect of ATP, not necessarily mediated by adenosine, as the final common pathway of changes in vascular tone in response to signals from the JGA and macula densa. A recent study demonstrates this using ratiometric calcium imaging of the in vitro microperfused isolated JGA-glomerular complex dissected rabbits (179).

New Directions

Fetal Programming for Hypertension: Failure of renoprotection?

The association of low birth weight with the development of hypertension later in life has been validated epidemiologically (180) and, more recently, has been demonstrated experimentally in animal models (181). A suboptimal fetal environment may lead to maladaptive responses, including failure of renal autoregulation and the development of hypertension. A reduction in nephron number during development may contribute to a reduction in GFR, but this is not always borne out in experimental studies. Multiple factors may contribute to the development of hypertension, but we will restrict ourselves to the role of renal hemodynamics. The RAAS may play a more important role as it is expressed early and associated with nephrogenesis. Blockade of the AT₁ receptor during the nephrogenic period after birth in the rat led to a decrease in nephron number, a reduction in renal function and hypertension (182). Protein restricted diets have also shown a rise in ACE levels in pregnant ewes (183) and a general stimulation of all components of the systemic RAS in other studies in response to protein restriction, which is blocked by treatment with an ACE inhibitor or an AT₁ receptor blocker. Thus, the adverse environment in utero, which programs the fetus to develop hypertension could be critically linked to abnormalities in the RAS (182).

Unconventional Behavior of RAS Components

While the canonical scheme of activation of the RAS components, from renin to Ang II and its effects on AT receptors is well recognized, recent evidence of other effects merit discussion. Both Ang I and Ang II may lead to effects that are independent of, or even antagonistic to, the accepted effects of the RAS (96). ACE may also function as a 'receptor' that initiates intracellular signaling and influences gene expression (184). AT₁ and AT₂ receptors have been shown to form heterodimers with other 7-transmembrane receptors, and influence signal transduction pathways (185–188). Intracellular Ang II exerts effects on cell communication, cell growth and gene expression via the AT₁ receptor, but also has independent effects (189,190).

Clinical Aspects

As discussed earlier, the development of the kidney occurs during the first 35 days postconception in humans. The integrity of the RAAS is essential for normal development, and Ang II is essential for normal structural development of the kidney and collecting system. The complex interplay of GTB and TGF increases with gestational age. The excretory function of the kidney begins soon after clamping of the umbilical cord at birth and continues through the first year of life. It follows that the neonate is exquisitely sensitive to stressors, which activate the RAAS, and lead to profound oligoanuric states.

Ischemia and Asphyxia Generate Renin by Sympathetic Stimulation

Drugs such as furosemide increase distal delivery of sodium and water, but reduce Na⁺K⁺2Cl activity, which should decrease renin release. However, decreased cotransporter activity impairs the TGF and, in the face of continued ion and water excretion, may result in hypovolemia, activation of the RAS and may lead to anuria. Indomethacin, used to treat patent ductus arteriosus in the neonate, could lead to altered renal vasoregulation (191). Congenital abnormalities in steroid synthesis could lead to deficiencies in aldosterone production, and cause profound salt-wasting states (192). In pseudohypoaldosteronism Type 1, mutations in the sodium channel may cause profound neonatal salt wasting (193). To counter the heightened awareness of the RAAS in neonates, adenosine receptor antagonists may offer new avenues of therapy.

SUMMARY

In summary, the RAAS is an important developmental and physiological system that contributes to renal blood flow and GFR autoregulation. Together with the myogenic response, the RAAS serves to maintain volume homeostasis in sodium and volume depleted states, and renoprotection in hypertensive states. The relative contributions of these two systems continue to be an area of controversy and debate. We also know that central and peripheral sympathetic input influences all aspects of renal autoregulation. More studies in transplanted or denervated kidneys in animal models and humans will elucidate these areas of controversy. A better understanding of these mechanisms would translate to better care for premature and full term newborns with renal dysregulation, hyponatremia and oligoanuric renal failure.

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