Chapter 11
The Classical and Nonclassical Renin-Angiotensin-Aldosterone System in Liver Cirrhosis

Giovanni Sansoè and Florence Wong

Abstract In chronic liver disease, excess hepatic deposition of extracellular matrix, the ensuing development of cirrhosis, the associated renal dysfunction, which ranges from pre-ascitic sodium retention to hepatorenal syndrome, are all dependent, to large extent, on altered function of the renin-angiotensin-aldosterone system (RAAS). The RAAS, once believed to be a hormonal system for blood pressure control and extracellular fluid volume regulation, is now considered a flexible and branching network of enzymes, peptides and receptors that regulates, in addition to arterial circulation, local and systemic inflammation, development of fibrosis in several organs, tumorigenesis, and even bodily reactions to common viruses. In patients with liver disease, besides production of angiotensin II by angiotensin converting enzyme in the vessel wall, there are adaptable synthesis and degradation of bioactive peptides within several tissues by means of enzymes that may be different from those located in arterial endothelium and smooth muscle cells. These 'nonclassical' RAAS metabolic pathways that lead to arterial vasodilatation, increased natriuresis and blunting of inflammation (e.g. through angiotensin 1-7 or 3-8) have been identified and can be manipulated pharmacologically, with foreseeable advantages in the treatment of circulatory and renal complications of liver cirrhosis and portal hypertension. Therefore, a comprehensive understanding of the classical and nonclassical RAAS pathways together with the enzymes and peptides involved, especially those that operate inside the liver and the kidney, will provide insights into disease pathogenesis and help to devise treatment strategies for the various disease processes.

Keywords Renin angiotensin system · Liver cirrhosis · Sodium retention · Liver fibrosis · Aldosterone · Chymase · ACE · ACE2 · Neprilysin

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Introduction

Ever since Tigerstedt and Bergman observed in 1898 that an extract of canine kidney, which they called renin, led to increased arterial blood pressure when injected into another animal, a challenge has been presented to scientists and renewed many times over [1]. The dilemma facing the investigators was not elucidated until 1940, when Prinzmetal showed the presence of renin in ischemic kidneys. Concepts were made clearer when Braun-Menendez identified the octapeptide, angiotensin II (Ang II), as the actual vasoactive compound leading to increased arterial pressure through its binding to a single cell membrane receptor, later called angiotensin type 1 receptor (AT_1R) [1]. Soon the 'classical' renin-angiotensin-aldosterone system (RAAS) was characterized (Fig. 11.1), only to become, over the following decades, less important as many other 'nonclassical' pathways of the RAAS were identified (Fig. 11.2) through the discovery of many more critical enzymes capable of producing a further series of angiotensin peptides. These peptides of different lengths, most of which endowed with specific functions, interact with at least four, and maybe five, different cell membrane receptors.

The challenge in this review is to explain concisely the role of the new and expanded RAAS in relation to the development of chronic liver disease up until the end stage of liver insufficiency, along with the related progressive derangement

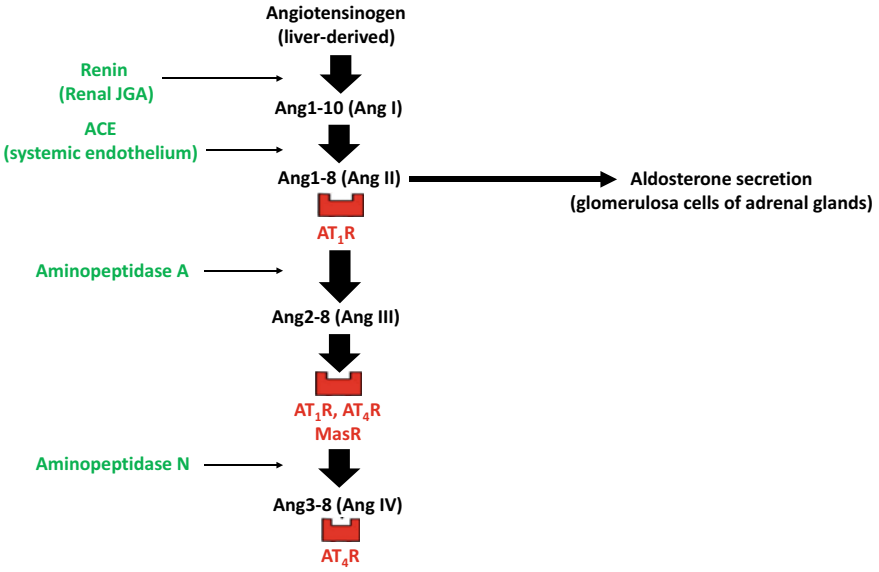

Fig. 11.1 Schematic representation of 'classical' renin-angiotensin-aldosterone system, with peptide receptors. Ang: angiotensin; AT_1Rs: angiotensin type1 receptors; AT_4Rs: angiotensin type4 receptor; MasR: Mas receptor, ACE: angiotensin converting enzyme; Ang: angiotensin; AT_1Rs: angiotensin type1 receptors; AT_4Rs: angiotensin type4 receptor; JGA: juxtaglomerular apparatus

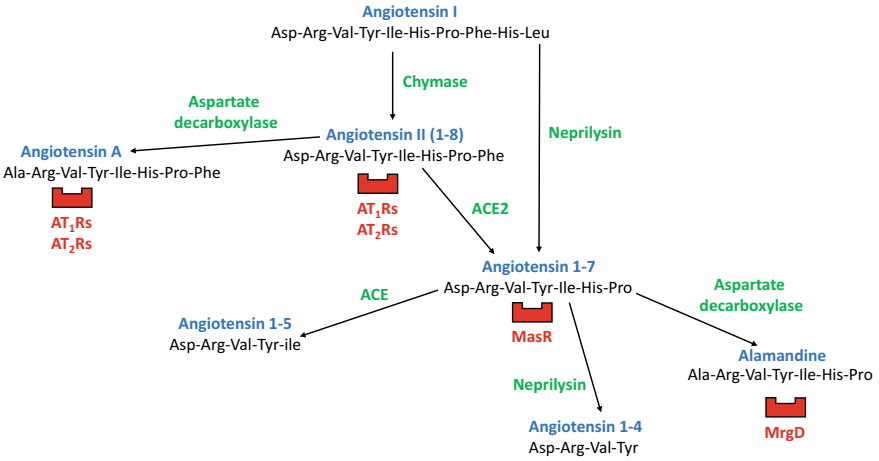

Fig. 11.2 Schematic representation of the 'non-classical' renin-angiotensin system, with peptide receptors. ACE: angiotensin converting enzyme; AT_1Rs: angiotensin type1 receptors; AT_2Rs: angiotensin type2 receptor; MasR; Mas receptor; MrgD: Mas-related G protein-coupled receptor member D

of renal and circulatory function that ensues. Several aspects of this system are worthy of note: (a) This complicated endocrine, paracrine, and even intracrine (i.e. intracellular) system of bioactive peptides of RAAS is now known for being deeply involved not only in the control of arterial blood pressure, as once was exclusively believed. It is also involved in the development of local and systemic inflammation, extracellular matrix deposition (fibrogenesis) in several organs, tumorigenesis, and even bodily reactions to common, sometimes lethal, viruses such as hRSV, SARS-CoV and SARS-CoV-2. (b) Many enzymes we will discuss (Figs. 11.1 and 11.2) can adapt their synthetic function when manipulated pharmacologically. Examples of such variable functional capability are shown in several alternative metabolic pathways: when angiotensin converting enzyme type 2 (ACE2) is blocked by metallopeptidase inhibitors, neprilysin starts converting Angiotensin I (Ang I) into angiotensin 1-7 (Ang1-7), the usual peptide product of ACE2 itself [2, 3]; when angiotensin converting enzyme (ACE) is blocked by ACE inhibitors (ACEis), chymase and cathepsin G start producing Ang II, with the result of a paradoxical increase in aldosterone plasma levels ('the aldosterone escape' phenomenon) during prolonged ACEi administration [3]; when renin is blocked by specific non-peptide inhibitors like aliskiren, Ang I and II are cleaved by chymase from the newly described angiotensins 1-25 (Ang1-25) and 1-12 (Ang1-12), which are polypeptides generated through non-renin pathways [4]. (c) These various enzymatic reactions sometimes occur in the systemic circulation (i.e. in the arterial endothelium and smooth muscle cells), sometimes in the local circulation (e.g. in heart, liver and kidney), sometimes in single cells that both produce and react to a peptide, sometimes inside the cytoplasm of definite cells (e.g. in cardiomyocytes). (d) Finally, these complex systems of 'classical' and 'nonclassical' systems (all cleaved from liver-derived α_2 globulin angiotensinogen)

can interfere with other endocrine or paracrine systems such as those of endothelins, kinins, plasmins, and with the secretion and function of catecholamines themselves [1].

In this review, we shall try to summarize all of this in relation to the development of chronic liver disease, mostly liver cirrhosis, and the cirrhosis-associated derangement of kidney function. Introduction to the basic physiology of the many protagonists of RAAS is essential to understand their perturbation in the setting of liver cirrhosis.

Physiology Considerations

(a) *Prorenin and its receptor*

The triggers to renin release are hypoperfusion of afferent glomerular arterioles, low chloride content in the macula densa segment of the nephron and stimulation of β_1 adrenergic receptors [5]. Losing a 43-amino acid N-terminal segment, a variable amount of the precursor prorenin is cleaved into the protease renin in the juxtaglomerular (JG) cells of the kidney, which are the only cells secreting active renin into blood, but a remarkable share of integral prorenin is also secreted into blood by the same JG cells and by adrenal glands [6], so much so that, normally, circulating prorenin levels are ten times higher than plasma renin concentrations. This ratio increases further in patients with diabetes and arterial hypertension [7]. One quarter of the circulating prorenin is proteolytically converted into renin in plasma by cathepsin B and proconvertase I or undergoes nonproteolytic activation into mature renin by binding to cell surface prorenin/renin receptors [(P)RRs]. Finally, half of circulating prorenin binds to nonspecific clearance receptors [8, 9] (Fig. 11.3).

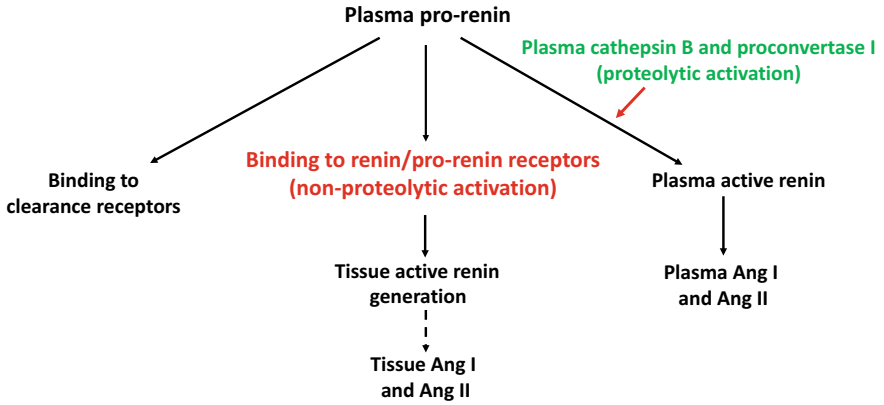

Fig. 11.3 Proteolytic and non-proteolytic activation of plasma prorenin into active renin. AngI: angiotensin I; AngII: angiotensin II

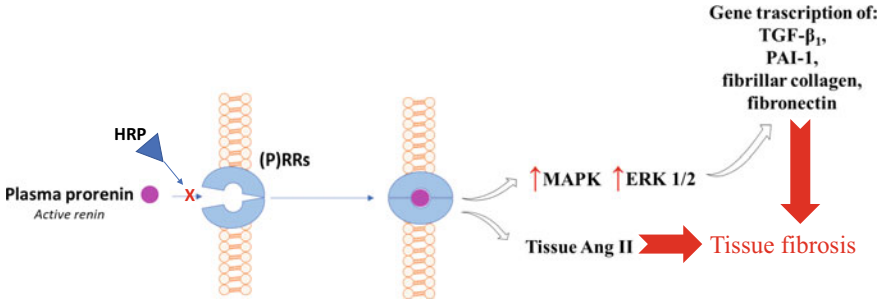

Fig. 11.4 Mechanisms of prorenin-induced tissue fibrogenesis. Agn II: angiotensin II; ERK 1 and 2: extracellular signal-regulated kinase 1 and 2; HRP: handle region of prorenin [(P)RR blocker]; MAPK: mitogen-activated protein kinase; PAI-1: plasminogen activator inhibitor-1; (P)RRs: tissue prorenin receptors; TGF-β_1: Transforming growth factor β_1

(P)RR is a 350-aminoacid protein that shows higher affinity for prorenin rather than active renin (Fig. 11.4). It is expressed in vascular smooth muscle cells and in mesangial, distal convoluted tubule and collecting duct cells of the kidney. In the distal nephron, (P)RRs are functionally associated with H^+-ATPases: these proton pumps transport protons across plasma membranes in the intercalated cells of collecting ducts and acidify urine in exchange with aldosterone-dependent Na^+ reabsorption [9].

Prorenin binding to (P)RRs promotes generation of Ang II in tissues. Independently of local Ang II, (P)RRs stimulation directly causes activation of stress related kinases such as mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK) 1 and 2, which upregulate transcription of pro-fibrogenic genes such as TGF-β_1, plasminogen activator inhibitor-1 (PAI-1), fibrillar collagen and fibronectin [10–12] (Fig. 11.4). Interestingly, estrogens increase ERK 1 and 2 phosphorylation and function through the same MAPK-dependent mechanism [13].

The peptide known as the 'handle region of prorenin' (HRP) prevents prorenin/renin binding to (P)RR (Fig. 11.4). Therefore, in experimental murine diabetes, HRP infusion reduces the glomerulosclerosis index and the renal content of TGF-β_1 and Ang II [14].

(b) *Role of calcium in renin secretion and renal sodium metabolism*

Pressor responses to sodium chloride loading in salt-sensitive essential hypertensive patients are preceded by a decrease in serum total and ionized calcium [15], and hypocalcemia with secondary hyperparathyroidism promotes arterial hypertension in chronic renal insufficiency [16]. Conversely, activation of the vitamin D receptor by 1,24-$(OH)_2$ vitamin D, which increases serum Ca^{++} concentrations through augmented intestinal absorption and decreased urine excretion of Ca^{++}, reduces renin secretion and indirectly sodium tubular retention [17]. Once again, the MAPK/ERK 1/2 pathway, notably stimulated by estrogens, was demonstrated to upregulate vitamin D receptors and therefore blunt renin secretion [13].

This means, as mentioned above, that fibrotic and sodium-retentive mechanisms might be regulated in opposite directions through the same sex hormone-dependent mechanism.

Plasma hypercalcemia stimulates the plasma membrane-associated receptors for extracellular calcium (calcium-sensing receptors or CaSR) in kidney JG cells, thus decreasing prorenin gene transcription and renin release through inhibition of adenylate cyclase, stimulation of phospholipase C, and production of diacylglycerol and inositol 1,4,5-triphosphate [18, 19]. CaSR stimulation by hypercalcemia also suppresses gene transcription and expression of vasopressin-dependent water channels in the kidney collecting duct [20] and reduces the content of sodium–potassium-chloride co-transporters in the thick ascending limb of the Henle's loop [21]. In other words, extracellular calcium acts as a natriuretic and diuretic agent.

(c) *Key endopeptidases and peptides of RAAS*

The 13-amino acid N-terminal sequence of angiotensinogen in humans is essential to understand the role of all RAAS peptidases [22]: N-Asp_1-Arg_2-Val_3-Tyr_4-Ile_5-His_6-Pro_7-Phe_8-His_9-Leu_{10}-Val_{11}-Ile_{12}-His_{13}…-C. Four different peptidases are the source of most bioactive peptides of the RAAS. The four peptidases are: ACE, a peptidyl-dicarboxypeptidase (EC 3.4.15.1 according to the EC system); ACE2, a peptidyl-monocarboxypeptidase (EC 3.4.17.23); chymase, a serine endopeptidase (EC 3.4.21.39); neprilysin, a Zn-metallo-endopeptidase (NEP, neutral endopeptidase, EC 3.4.24.11). ACE, ACE2 and NEP are classified as metallopeptidases. These four enzymes have membrane-anchoring domains that orient their active sites on the extracellular surface of the cell [23].

ACE. Once renin has cut the Leu_{10}-Val_{11} peptide bond of angiotensinogen to generate Ang I (Ang 1-10), dicarboxypeptidase ACE cleaves the Phe_8-His_9 bond of the decapeptide to make Ang II (Ang 1-8). ACE also cleaves the newly described angiotensin 1-12 (Ang1-12) into Ang I, angiotensin 1-9 (Ang1-9) into angiotensin 1-7 (Ang1-7) and, finally, Ang1-7 into inactive angiotensin 1-5 (Ang1-5) [24, 25]. Outside RAS, ACE degrades enkephalins, substance P and luteinizing hormone releasing hormone [26]. Kidney ACE is found in most tubular cells, vascular endothelial cells and glomerular mesangial cells. Outside the kidney, ACE is located in endothelial cells, especially in the lung [24].

ACE2. This monocarboxypeptidase is the main source of the vasodilator and natriuretic peptide Ang1-7 since it cleaves the Pro_7-Phe_8 bond of Ang II almost ubiquitously. Inside non-classical pathways of RAAS, ACE2 cleaves Ang I into Ang1-9 [24, 25]. ACE2 metabolizes also other peptide substrates (apelin, kinins and endorphins) and regulates the level of tryptophan in the blood [27].

NEP. This enzyme is a membrane-bound Zn-metallo-endopeptidase. It is also called atriopeptidase because it leads to the proteolytic clearance of urodilatin, atrial, brain-derived and C-type natriuretic peptides in the kidney, lung, brain, and heart [28]. NEP degrades opioid-peptides [29], bradykinin [30], bombesin-like peptide [31], substance P [32] and adrenomedullin [33]. Inside the RAAS, NEP cleaves Ang1-9, Ang1-12 and Ang I into Ang1-7 and, finally, the latter vasodilator and

natriuretic peptide into the inactive by-product angiotensin 1-4 (Ang1-4) [24, 25]. In other words, NEP generates Ang1-7, but continues to metabolize Ang1-7 at the Tyr_4-Ile_5 bond into Ang1-4 (Fig. 11.2), as shown in studies employing vasopeptidase inhibitors (i.e. combined ACE and NEP inhibitors) in patients or animal models with arterial hypertension [34, 35].

NEP also produces the vasoconstrictor, profibrogenic and anti-natriuretic polypeptide endothelin-1 (ET-1) from circulating precursors (big ET-1 and ET 1-31) [36, 37].

Chymase. Serine endopeptidase chymase, in heart, liver, renal tubules and mast cells, converts Ang I into Ang II; ACE catalyzes this same reaction in the arterial endothelium [38]. In tissues with chronic inflammation, chymase is over-expressed and converts big ET-1 into ET-1 [39] and activates TGF-β_1 through potentiation of Ang II action [40]. 80% of Ang II synthesized in kidney, heart and blood vessels is produced by chymase [41], but chymase inhibitors do not lower blood pressure and do not increase active renin [42] because chymase is confined to mast cells of the vascular adventitia of arterial vessels. Moreover, systemic plasma contains serine endopeptidase inhibitors [42]. Chymase also cleaves Ang1-12 into the Ang II. Upstream in this atypical metabolic pathway, Ang1-12 is not cleaved from angiotensinogen by renin, but through a hitherto unidentified protease that cuts the Ile_{12}-His_{13} bond of angiotensinogen in humans [43, 44]. Ang1-12 may be a precursor of Ang I through ACE [45, 46], of Ang1-7 and then Ang1-4 (in the tubular nephron) through NEP [46], and, above all, of Ang II through chymase in heart and kidney [43]. Recent studies underline that, alongside Ang1-12, another polypeptide derived from angiotensinogen through non-renin pathways (i.e. angiotensin 1-25) may be a suitable source of tissue Ang I and II by means of chymase action, at least in the heart [47, 48].

(d) *Angiotensin receptors so far described*

The cell surface receptors identified so far as binding sites of this host of angiotensins are five: $AT_{1\text{-}2\text{-}4}$Rs, Mas receptors (MasRs) and Mas-related G protein-coupled receptor member D (MrgD) (Figs. 11.1 and 11.2). The main endogenous ligand of AT_1R and AT_2R in vascular endothelium, kidney, adrenals, brain, heart, liver and testis is Ang II. Ang 1-9 is also a ligand of AT_2R (7). The main endogenous ligands of AT_4R and MasR are, respectively, angiotensin 3-8 (Ang3-8) and Ang1-7 [25]. Putative ligand of MrgD is newly described heptapeptide alamandine (Fig. 11.2).

AT_1 receptors. AT_1Rs greatly exceed the number of AT_2Rs after birth [25], leading to vasoconstriction, aldosterone secretion from the glomerulosa cells of adrenal glands, tubular sodium retention and increased arterial blood pressure, when stimulated by Ang II (classical RAAS).

AT_1R signaling is primarily mediated through G-proteins, leading to adenylyl cyclase activation and intracellular cAMP generation, activation of phospholipase C, production of inositol-1,4,5-triphosphate (IP3), Ca^{++} release from sarcoplasmic reticulum into the cytoplasm, and final Ca^{++}/calmodulin-dependent vasoconstriction. Further AT_1R signaling is mediated through small GTPase proteins, G-protein independent β-arrestin, reactive oxygen species (ROS) (through NADPH-oxidase [NOX]

activation, leading to tissue fibrogenesis) [49], non-receptor type tyrosine kinases, transactivation of receptor tyrosine kinases [50]. AT_1Rs also undergo homo and hetero oligomerization with other receptors, including AT_2Rs, bradykinin B_2 receptors, β_2 adrenergic receptors, and dopamine D_2 receptors [51]. Recently, it has been shown that AT_2Rs directly bind to AT_1Rs, inhibiting AT_1R functions. Bradykinin B_2 receptors potentiate AT_1R signaling, enhancing the vasoconstrictive effects of Ang II. Evidence also exists of direct interaction between the β_2-adrenergic receptors and AT_1Rs. β-blockers have been shown to interfere with Ang II signaling in heart failure patients and have become a mainstay of therapy in patients with chronic heart failure [52].

AT_2 receptors. These G protein-coupled receptors share only 34% amino acid sequence homology with AT_1Rs [53]. AT_2R signaling involves G protein, specific protein phosphatases (MKp-1, PP_2A, etc.) and scaffolding proteins, nitric oxide/cGMP ion channel protein, and constitutive activity (i.e. ligand independent activity of AT_2R) [50]. Stimulation of AT_2Rs in interlobular arterioles and the tubular nephron of the kidney leads to vasodilatation and natriuresis. The latter effect is mediated by stimulation of nitric oxide/cGMP/Sp 1 pathways that inhibit the proximal tubule Na^+/K^+-ATPase [25, 54]. Moreover, it appears that specific stimulation of AT_2R can down-regulate expression of AT_1Rs, resulting in the finding that, based on the AT_1R/AT_2R balance, Ang II itself can be hyper- or hypotensive and natriuretic or anti-natriuretic [54, 55].

AT4 receptors. AT_4Rs are mainly in brain, heart, kidney, adrenals and blood vessels. This receptor is the Angiotensin 3-8 (Ang3-8) binding site (Fig. 11.1). Ang3-8 binding protein was identified as insulin-regulated amino peptidase (IRAP, EC 3.4.11.3), which is a type 2 trans-membrane protein of the gluzincin amino peptidase family [56, 57].

Mas receptors. MasR is a G protein-coupled receptor and the binding site for Ang1-7. The action of Ang1-7 through MasR causes production of arachidonic acid and activation of nitric oxide synthase. MasRs exhibit the highest expression in brain and testis [50]. In common with AT_4R, stimulation of vasodilator and natriuretic MasR leads to nitric oxide production via enhanced phosphorylation of protein kinase B and increased cell levels of cyclic GMP [24, 25].

Mas-related G protein-coupled receptor member D (MrgD). MrgD expression is detected in arterial smooth muscle cells, endothelial nitric oxide synthase (eNOS)-positive endothelial cells, and in atherosclerotic plaques [58]. MrgD stimulation is thought to elicit phospholipase C activation and increased expression of nitric oxide synthase (NOS) enzymes [58]. MrgD is the putative binding site for alamandine, an heptapeptide derived from Ang1-7 through decarboxylation of the N-terminal aspartate residue (Fig. 11.2).

(e) *Intracrine RAAS*

Over the last decades, a large amount of literature has shown that not only do tissue renin-angiotensin systems exist, but so do intracellular (i.e. intracrine) renin-angiotensin systems. Various reports have identified intracellular location and actions

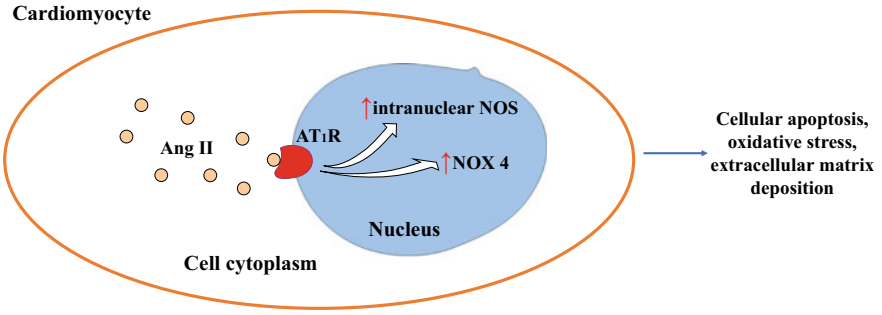

Fig. 11.5 Synthetic representation of intracrine RAAS inside cardiomyocytes. Ang II: angiotensin II; AT_1R: angiotensin type 1 receptor; NOS: nitric oxide synthase; NOX 4: NADPH oxidase 4

of such RAAS components as Ang II, Ang1-7, prorenin receptor, angiotensinogen, several isoforms of renin, AT_1Rs, AT_2Rs, MasRs, ACE, ACE2 and chymase [59]. Perhaps intracellular RAAS alone would warrant a separate review. What matters here may be summarized as follows. Ang II treatment produces a significant increase in nitric oxide (NO) and superoxide/H_2O_2 production in isolated nuclei (Fig. 11.5). These effects are inhibited by losartan (an AT_1R inhibitor) but not by an AT_2R blocker [60]. The likely sources of these intracellular NO and reactive oxygen species are intranuclear NOS and NADPH oxidase 4 (NOX 4). At least in diabetic rats, the intracellular Ang II content in the heart is correlated with cardiomyocyte apoptosis, oxidative stress and extracellular matrix deposition [61] (Fig. 11.5). There is strong experimental evidence to support the view that intracrine Ang II activity may function independent of the circulating RAAS [47]. Whether these findings can be transferred to the model of liver fibrosis is a matter of debate.

(f) *Aminopeptidases and Ang II clearance*

In the systemic circulation, degradation of Ang II may lead to Ang1-7 generation through ACE2, but, in wild-type mice and normal humans, low systemic levels of Ang1-7 and much higher levels of angiotensin 2-8 (Ang2-8 or Ang III) and angiotensin 3-8 (Ang3-8 or Ang IV) [62] emphasize that the actual clearance of Ang II is through the sequential actions of plasma aminopeptidases (Fig. 11.1). In plasma, aspartyl-aminopeptidase or aminopeptidase A (APA) cleaves the Asp_1-Arg_2 bond at the N-terminal end of Ang II to generate Ang2-8, which in turn is cleaved at the new N-terminal Arg-Val bond by arginyl-aminopeptidase or aminopeptidase N (APN), to form Ang3-8 [25]. The kidney synthesizes and secretes most APA and APN found in blood [62, 63].

Ang2-8 and Ang3-8, have important hormonal activities. Ang2-8 binds AT_1R, AT_2R and MasR, and Ang3-8 binds mostly AT_4R. AT_4R stimulation by Ang3-8 causes arterial vasodilatation and natriuretic responses [25].

In summary, all RAAS peptides that are generated downstream of Ang II are either vasodilating and natriuretic agents (Ang1-7, Ang3-8, alamandine and even Ang2-8 when stimulating AT_2Rs or MasR) or inactive by-products (Ang1-5, Ang1-4) (Figs. 11.1 and 11.2).

Liver Cirrhosis. Prorenin and Renin Regulation by Extracellular Calcium

By comparing normal and CCl_4 cirrhotic rats, it is found that (P)RR content in the liver is significantly lower, not higher, in the cirrhotic group (western blot analysis). Conversely, plasma concentrations of prorenin can be derived empirically from the ratio direct renin (DR)/plasma renin activity (PRA) [1], and DR/PRA ratios were 3.3 $\pm$ 0.8 and 7.9 $\pm$ 1.6 ($P < 0.03$) in healthy and cirrhotic rats respectively, showing more plasma prorenin in the latter group [64]. Significantly lower content of (P)RRs in the cirrhotic liver along with increased circulating prorenin may be the expression of physiological receptor downregulation after prolonged agonist stimulation. To summarize, it is clear that (P)RR is expressed also in the liver and, as such, its role as a pathogenic factor, among many others, of hepatic fibrogenesis cannot be excluded (Figs. 11.3 and 11.4).

Sansoè and Wong observed significant natriuretic and aquaretic responses to intravenous calcium loading in human compensated cirrhosis [65] and to intravenous administration of CaSR agonists (i.e. poly-L-arginine) in experimental pre-ascitic cirrhosis [66]. Of course, these calcium-driven diuretic responses were not accompanied by any increase in plasma renin activity, due to the already described downregulating effects of CaSR stimulation on renin gene transcription and secretion by JG cells [18, 19].

Liver Cirrhosis. Endopeptidases and Peptides of RAAS Are Protagonists in Chronic Liver Disease and Its Renal Complications

Within the liver, low levels of ACE activity are detected in sham/control animals, while significantly increased levels are shown in areas of active fibrogenesis in bile duct ligated or CCl_4-treated rats [67–69]. Inhibition of ACE reduces increased arterial blood pressure, and ACE inhibitors (ACEis) or AT_1R antagonists (ARBs) can attenuate experimental liver fibrosis [68, 70], but these two classes of drugs have severe hypotensive effects in patients with established cirrhosis [71, 72]. Concentrations of ACE2, Ang1-7 and MasR (Ang1-7 specific receptor) (Fig. 11.2) are increased in splanchnic vessels from cirrhotic patients and rats compared to healthy controls [73]. Therefore, MasR blockade reduces portal pressure, indicating that activation of this

receptor in splanchnic vasculature promotes mesenteric hyperdynamic circulation and increases portal inflow that contributes to portal hypertension [73]. However, non-peptidic MasR agonist AVE0991 reduces portal pressure without any change in arterial blood pressure [74]. At first sight, these data seem contradictory: it was apparently shown that both MasR blockers and MasR agonists reduce portal pressure. It is conceivable that MasR blockade reduces portal venous inflow, as stated, while MasR agonists reduce intrahepatic resistance to portal flow since ACE2 is upregulated in areas of active liver fibrogenesis [75]. As a matter of fact, recombinant ACE2 has anti-fibrogenic effects in bile duct ligated (BDL) and CCl_4-treated rats, both acutely and long-term [76, 77], and diminazene aceturate, commonly used to treat human trypanosomiasis, enhances hepatic ACE2 activity and inhibits tumor necrosis factor-α (TNF-α) synthesis and gene expression of NADPH oxidase (NOX), a key source of fibrogenic reactive oxygen species (ROS) [78]. So doing, this drug exerts strong hepatic anti-fibrotic properties. ACE2, indeed, is thought to be a negative regulator of the RAAS and, in the liver, this enzyme functions to limit fibrosis [79].

In the kidney of cirrhotic rats with ascites, there is a mean 170% increase in NEP protein content, and NEP localizes mainly in proximal convoluted tubule and macula densa [80]; the NEP inhibitor candoxatrilat promptly increases urinary volume, and urinary excretion of sodium, atrial natriuretic peptide (ANP) and cyclic GMP (ANP second messenger), without significant changes in plasma renin activity or mean arterial pressure [80]. These overall results depend on the key contribution of NEP to ANP, Ang1-7, bradykinin clearance and to tissue ET-1 generation [24, 25, 28]. Notably, in patients with cirrhosis and ascites, renal plasma flow (RPF) and glomerular filtration rate (GFR) inversely correlate with plasma levels of ET-1 [81], and intravenous infusion of ET-1 results in prompt anti-natriuretic responses [82]. In the cytosol fraction of the cirrhotic rat liver, there is a even greater increase in NEP content, 280% to be exact. This enzyme is in the desmin-positive myofibroblast-like cells of the fibrotic septa. NEP inhibitor candoxatrilat, administered to rats with CCl_4-dependent cirrhosis, acutely decreases portal pressure and increases liver plasma flow (evaluated through indocyanine green clearance) [83]. In the kidney of CCl_4 cirrhotic animals, chymase protein content and activity are significantly increased in cortical arterioles and the tubular nephron. In cirrhotic rats and hamsters, chronic dosing of SF2809E, a specific chymase inhibitor, decreases renal Ang II content and increases natriuresis and aquaresis [84, 85]. In the liver of CCl_4 cirrhotic rats, chymase is largely expressed in α-smooth muscle-positive myofibroblasts, while, in human cirrhosis, chymase is mainly found in hepatocytes of regenerative nodules. Moreover, chymase mRNA transcription is promptly upregulated by TGF-β_1 in human HepG2 cells and activated hepatic stellate cells in vitro. Finally, SF2809E, specific chymase inhibitor, reduces liver Ang II content, hepatic fibrogenesis and portal pressure in CCl_4-treated animals [84, 85].

To sum up, in the diseased liver, areas of active fibrogenesis express increased contents of ACE [67], chymase [85], and NEP [83], but also of ACE2 [75]. This leads to increased tissue levels of Ang II [85] and some five-fold increase in the Ang II/Ang1-7 ratio in the diseased liver [64]. Particularly critical is the role of desmin- and α-smooth muscle-positive liver myofibroblasts (HSC/MFs) of liver fibrotic septa:

these cells host over-expressed NEP [83], the enzyme that degrades the vasodilating and anti-fibrogenic Ang1-7 [24, 25], and are active sources of Ang II and ET-1 through cellular over-expression of ACE and chymase [69, 85].

Moreover, in patients with liver cirrhosis, renal RAAS is aberrantly activated: angiotensinogen is secreted into proximal tubular fluid [25], active renin is massively produced in advanced cirrhosis with ascites, and ACE, NEP and chymase are upregulated and hyperactive in the tubular nephron even before clinical decompensation [24, 25, 83, 85]. This leads to Ang II concentrations in the kidney interstitial and tubular fluids being much higher than normal well before ascites development and before secondary hyper-reninism (i.e. irrespective of systemic levels of Ang II) [25, 85], producing a net effect of sodium retention along all segments of the tubular nephron (see later for the specific mechanisms of Ang II-dependent renal sodium retention).

Liver Cirrhosis. Receptors of Angiotensins and Post-receptor Mechanisms of Disease

Ang II is a key contributor (through binding to AT_1Rs) to progression of liver fibrogenesis, cirrhosis development, and worsening of hepatic function in chronic liver disease. Liver fibrosis progression depends on interactions among injured hepatocytes, activated inflammatory cells, and hepatic myofibroblast (MFs)-like cells that originate mainly from activation of hepatic stellate cells (HSCs) or portal fibroblasts. Activated HSCs produce Ang II [67], Ang II binds to AT_1R expressed by most myofibroblasts, and transcription of genes encoding for extracellular matrix components, pro-fibrogenic cytokines (e.g. TGF-β_1) and collagenolysis inhibitors occurs [86–88].

The role of AT_1R signaling in HSC activation and collagen deposition in chronically diseased liver is predominant (Fig. 11.6).

- *RhoA/Rock-1 pathway*. Among members of the Rho small GTPase superfamily (AT_1R signaling mediators), Ras homolog gene family member A (RhoA) constitutes the RhoA/Rock-1 (Rho-associated coiled-coil-containing kinase protein-1) signaling pathway, with resultant activation of the small G protein Rac and reactive oxygen species (ROS) production, which plays a central role in the development of liver fibrosis [89]. Notably, one of the most important effects of ROS is the reduction of nitric oxide (NO) bioavailability: superoxide radical anion (O_2^-) reacts with NO, destroying it via its conversion to peroxynitrites [90] (Fig. 11.6). In BDL rats, liver collagen deposition can be blunted and portal pressure decreased through inhibition of the RhoA/Rock 1 signaling pathway, which is instead activated by Ang II through AT_1R, [91].
- *JAK2 pathway*. Through mechanisms that are not fully understood but probably involve Ca^{++} and PYK_2 or Src kinase, stimulation of AT_1Rs activates intracellular Janus kinase-2 (JAK2). JAK2 then phosphorylates Rho guanine nucleotide exchange factor 1 (ARHGEF1), which stimulates the RhoA-Rho kinase–myosin

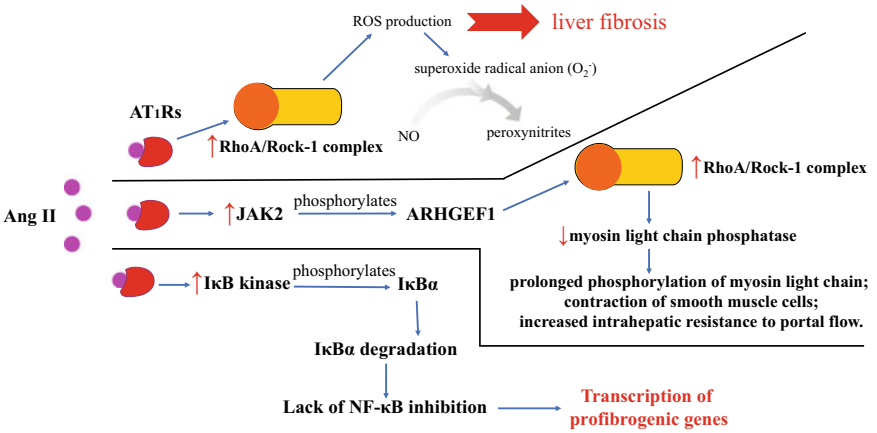

Fig. 11.6 Mechanisms of liver disease mediated through AT_1R stimulation by Ang II. Ang: angiotensin; AT_1R: angiotensin type 1 receptor; ARHGEF1: Rho guanine nucleotide exchange factor 1; IκB: inhibitor of NF-κB; JAK2: intracellular Janus kinase-2; NF-κB: Nuclear factor κB; NO: nitric oxide; RhoA: Ras homolog gene family member A; Rock-1: Rho-associated coiled-coil-containing kinase protein-1; ROS: reactive oxygen species

phosphatase target subunit cascade, and this inhibits myosin light chain phosphatase, leading to prolonged phosphorylation of myosin light chain and final contraction of vascular smooth muscle cells, the physiological effect of Ang II [92] (Fig. 11.6). Interestingly, JAK2 antagonists significantly attenuate HSC activation and collagen accumulation in experimental liver fibrosis models [93]. Notably, JAK2 phosphorylates and activates signal transducer and activator of transcription 3 (STAT3), and JAK2/STAT3 pathway is aberrantly expressed in tissues infected by SARS-CoV-2 during COVID-19 [2, 94], as well as in most malignancies: e.g., breast, pancreatic, bladder, colorectal, gastric cancers, lung adenocarcinoma, and natural killer/T-cell lymphoma [95].

- *NF-κB pathway*. Activated liver HSCs express constitutive nuclear factor-κB (NF-κB), which promotes HSC survival by stimulating the expression of anti-apoptotic proteins. Specific inhibition of NF-κB is sufficient to provoke apoptosis of mature human HSCs and blunting of liver collagen deposition. Human HSC activation is accompanied by a sustained transcriptional repression of IκBα, the natural inhibitor of NF-κB. Moreover, upon stimulation of AT_1Rs in activated HSCs, serine residues on IκBα are phosphorylated by the IκB kinase. This results in progressive degradation of IκBα, which releases NF-κB for nuclear transport and interaction with profibrogenic target genes, leading to their transcription [96] (Fig. 11.6).

In liver cirrhosis, intrarenal RAAS is activated earlier than its systemic counterpart, as confirmed in humans with pre-ascitic disease. In fact, lower-body negative pressure, which reduces central blood volume, enhances renal renin and Ang II secretion rates [97]. Moreover, despite baseline suppression of systemic RAAS, sodium

overload induced by high sodium diet is reversed by the AT_1R antagonist losartan administered at a dose not perturbing systemic hemodynamics, stressing exclusive intrarenal activation of renin-angiotensin system [98, 99].

In the kidney, Ang II constricts the efferent glomerular arteriole more than the afferent one, resulting in a tendency to preservation of GFR and filtration pressure. This occurs at the expense of reduction in renal plasma flow, increase in filtration fraction, and decrease in peritubular capillary hydrostatic pressure. The latter leads to retention of sodium and water in the tubular nephron [100].

In addition, Ang II causes direct sodium reabsorption in the proximal convoluted tubule through stimulation of tubular AT_1R and activation of renal cortical Na^+/H^+ exchanger 3, a process involving an increase in intracellular Ca^{++} and activation of JAK2 and calmodulin [100, 101]. Enhanced release of Ang II and increase in oxidative stress (through activation of NOX and RhoA/Rock 1 kinase pathways) are also the key to further renal sodium retention via increased activity of thiazide-sensitive sodium chloride cotransporter in the later segments of the distal convoluted tubule [90]. Finally, increased systemic levels of Ang II and secondary aldosteronism lead to aldosterone-dependent Na^+/K^+-ATPase and epithelial sodium channels (ENaCs) upregulation in the collecting duct and stimulation of arginine vasopressin (AVP) secretion. In turn, increased plasma AVP and increased reactive oxygen species (ROS) (due to stimulation of kidney AT_1Rs) enhance the activity of Na^+-K^+-$2Cl^-$ cotransporters in the thick ascending limb of the Henle's loop [90]. In other words, increased renal content of Ang II, firstly, and increased systemic levels of Ang II with secondary aldosteronism, secondly, completely control sodium retention along all segments of the tubular nephron in cirrhosis, both in pre-ascitic and in ascitic patients.

Unfortunately, in patients with cirrhosis and ascites or end stage liver disease, oral ACEis or ARBs, due to their arterial vasodilatory activity, do not improve natriuresis and may aggravate arterial hypotension and hyper-reninism, leading to final fall in both RPF and GFR [102–105]. This is due to the systemic activation of RAAS, which nonetheless tries to compensate for the peripheral arterial vasodilatation of advanced cirrhosis [102]. Perhaps compensated patients with early cirrhosis and no systemic RAAS activation might take advantage of ARBs administration, at least to reduce liver fibrogenesis [67, 103]. In any event, recent systematic reviews of available trials show that ARBs, in patients with ascitic cirrhosis, do not reduce portal pressure significantly and increase the risk of symptomatic hypotension and renal failure [1, 104]. Moreover, it has long been known that ACEis in liver cirrhosis do not reduce portal pressure in Child–Pugh A cirrhotic patients [105–107] and, of course, are detrimental in decompensated cirrhotic patients [108].

Finally, in clinical settings characterized by enhanced systemic production of Ang II (e.g. in decompensated cirrhosis), AT_1Rs-enriched exosomes transfer such receptors to peripheral target cells, in order to offset the physiological receptor downregulation after prolonged agonist stimulation. Exosomes are extracellular nanovescicles of 30–100 nm in size that are released into the extracellular space by cardiomyocytes through reverse budding of multivesicular intracellular bodies [109].

The discovery of non-peptidic AT_2R agonists offers hope for new therapeutic approaches to modify the AT_1R/AT_2R balance [110]. Among these AT_2R agonists, the most promising one, in relation to the management of cirrhosis complications, is Compound 21 (C21), which, in animal models of arterial hypertension, produces dose-dependent natriuretic and aquaretic effects but does not reduce blood pressure unless the AT_1Rs are also blocked [111].

Moreover, agonists of AT_2R do blunt fibrogenesis in chronic liver disease [55].

The putative MrgD ligand alamandine (Fig. 11.2) can attenuate arterial hypertension, alleviate cardiac hypertrophy in spontaneously hypertensive rat [58], and appears to attenuate hepatic fibrosis by regulating autophagy induced by NOX 4-derived reactive oxygen species [112]. Unfortunately, no human studies are available regarding this specific topic.

Liver Cirrhosis. Secondary Aldosteronism, Renal Sodium Retention and Progression of Liver Fibrosis

Patients with advanced liver cirrhosis and ascites display splanchnic and systemic hyperdynamic circulation, contraction of effective arterial blood volume, hyperreninism and secondary aldosteronism [113]. Beyond the expected worsening of sodium retention because of secondary hyperaldosteronism itself, aldosterone, whose secretion by glomerulosa cells of adrenal glands is under Ang II control through stimulation of AT_1Rs, has a definite role also in the initial development of cirrhotic ascites. In rats with CCl_4-induced cirrhosis, pre-ascitic renal sodium retention is temporally related with increasing renal aldosterone excretion and is prevented by the aldosterone antagonist spironolactone [114]. In upright pre-ascitic cirrhotic patients, renal sodium retention is associated with a borderline elevation in plasma aldosterone and increased tubular sodium reabsorption by the distal nephron [115].

This traditional view of aldosterone as a trigger of clinical decompensation of liver cirrhosis has been recently enriched after the observation that patients with arterial hypertension chronically treated with ACEis show paradoxically high levels of circulating aldosterone because of the so called 'aldosterone escape': when ACE is blocked by ACE inhibitors, chymase and cathepsin G start producing Ang II, with the result of increased aldosterone plasma levels during prolonged ACEi administration. This 'aldosterone escape phenomenon' is thought to be the cause of ACEi treatment failure in the prevention of progressive renal fibrosis that occurs in subgroups of patients with arterial hypertension. Indeed, sustained increased levels of plasma aldosterone, as occur also in patients with advanced liver cirrhosis, induce ubiquitous plasminogen activator inhibitor-1 (PAI-1) expression, and treatment with mineralocorticoid receptor antagonists reverses this phenomenon. PAI-1 is a member of the serine protease inhibitor (serpine) gene family and the main inhibitor of tissue-type and urokinase-type plasminogen activators (tPA and uPA), and therefore of fibrinolysis. Unfortunately, the same tissue PAI-1, as such induced by increased plasma levels

of aldosterone, is also a strong inhibitor of plasmin-dependent matrix metalloproteinases (MMPs) activation in the liver, where MMPs should provide the reabsorption of excess extracellular matrix deposition during chronic liver diseases [3, 116–118]. In brief, plasma aldosterone, through increased PAI-1 gene expression, is considered a relevant agent of progressive liver fibrosis in chronic liver disease [117, 118].

Liver Cirrhosis. Aminopeptidases and Chronic Liver Disease

Recently, it has been shown that plasma aminopeptidase A is significantly reduced in patients with liver cirrhosis [119]. In this clinical context, this means that lack of aminopeptidase A provides less Ang2-8 to aminopeptidase N, which in turn generates lesser amounts of the natriuretic Ang3-8 (Fig. 11.1). Furthermore, slowed degradation of Ang II itself means prolonged half-life of this key anti-natriuretic peptide, which perpetuates the vasoconstrictive and sodium retaining effects of Ang II.

Conclusions

With a more comprehensive understanding of the systemic and tissue RAAS, it is perhaps time to advance an updated theory of liver insufficiency and associated functional renal failure in cirrhosis. What was once thought of as secondary to mere hemodynamic abnormalities (i.e. the hyperdynamic circulation of liver cirrhosis with ensuing contraction of effective arterial blood volume) is now complementary to our understanding of the changes that occur both inside the diseased liver and inside the kidney: chymase, ACE, NEP are overexpressed and functioning in both organs, leading to a net imbalance towards too much Ang II and too little Ang1-7. The consequences in the organs are different: inflammation and progressive fibrosis inside the liver, vasoconstriction, tubular sodium retention and final GFR loss inside the kidney.

Conflicts of Interest None to declare.

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