Physiology of Local Renin-Angiotensin Systems

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I. Local Renin-Angiotensin Systems
A. Definition of local RAS
B. Components of the local RAS
II. Localization and Functional Aspects
A. Heart
B. Vasculature
C. Nervous system
D. Reproductive tract
E. Skin
F. Digestive organs
G. Sensory organs
H. Lymphatic tissue
I. Adipose tissue
III. Prenatal Development
IV. Summary

Paul, Martin, Ali Poyan Mehr, and Reinhold Kreutz. Physiology of Local Renin-Angiotensin Systems. Physiol Rev 86: 747–803, 2006; doi:10.1152/physrev.00036.2005.—Since the first identification of renin by Tigerstedt and Bergmann in 1898, the renin-angiotensin system (RAS) has been extensively studied. The current view of the system is characterized by an increased complexity, as evidenced by the discovery of new functional components and pathways of the RAS. In recent years, the pathophysiological implications of the system have been the main focus of attention, and inhibitors of the RAS such as angiotensin-converting enzyme (ACE) inhibitors and angiotensin (ANG) II receptor blockers have become important clinical tools in the treatment of cardiovascular and renal diseases such as hypertension, heart failure, and diabetic nephropathy. Nevertheless, the tissue RAS also plays an important role in mediating diverse physiological functions. These focus not only on the classical actions of ANG on the cardiovascular system, namely, the maintenance of cardiovascular homeostasis, but also on other functions. Recently, the research efforts studying these noncardiovascular effects of the RAS have intensified, and a large body of data are now available to support the existence of numerous organ-based RAS exerting diverse physiological effects. ANG II has direct effects at the cellular level and can influence, for example, cell growth and differentiation, but also may play a role as a mediator of apoptosis. These universal paracrine and autocrine actions may be important in many organ systems and can mediate important physiological stimuli. Transgenic overexpression and knock-out strategies of RAS genes in animals have also shown a central functional role of the RAS in prenatal development. Taken together, these findings may become increasingly important in the study of organ physiology but also for a fresh look at the implications of these findings for organ pathophysiology.

I. LOCAL RENIN-ANGIOTENSIN SYSTEMS

A. Definition of Local RAS

In its “textbook” definition, the renin-angiotensin system (RAS) is a peptidergic system with endocrine characteristics. The substrate of the system, angiotensigen, an α-glycoprotein, is released from the liver (152, 250, 444) and is cleaved in the circulation by the enzyme renin that is secreted from the juxtaglomerular apparatus of the kidney (245, 250, 540, 631) to form the decapeptide angiotensin (ANG) I. ANG I is then activated to the octapeptide ANG II by angiotensin converting enzyme (ACE), a membrane-bound metalloproteinase, which is predominantly expressed in high concentrations on the surface of endothelial cells in the pulmonary circulation (109, 111, 250, 486, 664, 665, 767). ANG II, considered the main effector peptide of the RAS, is then acting on specific
receptors, for example, to induce vasoconstriction by interacting with ANG receptors on vascular smooth muscle cells or by stimulating the release of aldosterone from the adrenal cortex (250, 285, 562).

This view of the RAS, which has been generated by accumulated evidence over decades, had to be expanded significantly by more recent findings that increased the complexity of the system. Different ANG receptors (AT₁, AT₂, AT₄) and signal transduction pathways involved have been characterized (142, 306, 462, 505, 604, 639, 691, 712, 724, 785). Moreover, additional truncated peptides such as ANG-(1—7) have been identified (200, 310, 420, 595, 769), and alternative pathways of ANG II formation, for example, by the serine protease chymase (which can also cleave ANG I to form ANG II), have been proposed (20, 728, 730, 781).

The resulting change of our view of the RAS has been the introduction of the concept of “local” or “tissue” renin angiotensin systems (178, 415, 527). This concept was based on findings of RAS components in “unlikely” places (such as the “kidney enzyme” renin in the brain) where the endocrine actions of the system could not explain the findings (219, 220). This in turn led to new hypotheses and functional concepts of local RAS actions based on the tissue-based synthesis of ANG II. It is not surprising that the notion of tissue-based RAS with independent actions was not received with great enthusiasm, but rather led to significant controversies on the subject. Nevertheless, the database on local RAS accumulated so far has by and large been convincing and strengthened by two major technical advances, namely, the use of molecular biology and the availability of transgenic and knock-out models with altered expression of RAS components (28, 31, 106, 370, 377, 534, 537, 696).

The genes for all components of the RAS have been cloned, and gene expression studies could verify their mRNA expression and regulation in many tissues, demonstrating the possibility of local ANG II synthesis. Over-expression of RAS genes in transgenic mice and rats as well as the knock-out of these genes in mice have allowed detailed studies on the function of local RAS (28, 31, 106, 370, 376, 534, 537, 696). It has also become increasingly clear that these systems are not isolated entities but can interact with the endocrine RAS as well as other peptide systems (such as the endothelin system) on multiple levels (538, 582, 585, 614).

The early controversy on the novel concept of tissue RAS has been based on the question of local synthesis versus uptake from the circulation. A case in point represents the controversy between investigators after the demonstration of renin expression in the heart (178, 415) and those questioning local renin synthesis (751). This controversy was based on the fact that detection of renin mRNA in the heart could only be demonstrated inconsistently, leading to the suggestion that studies measuring cardiac renin were based on artifacts due to contamination with plasma renin or active renin uptake from the circulation. This issue should not threaten the concept of local RAS, since either mechanism could contribute to local ANG synthesis and actions. Modern concepts of the tissue RAS, therefore, are function oriented.

B. Components of the Local RAS

The characteristics and regulatory significance of long-known components of the tissue RAS such as renin, angiotensinogen, ACE, and ANG peptides (ANG I and II) have been reviewed extensively (27, 172, 177, 381, 415, 482, 527, 727) and will not be addressed further in this section. The focus of this section is set rather on recent discoveries concerning new factors and pathways involved in ANG biosynthesis and function.

1. Prorenin and renin binding and receptors

The binding of prorenin and renin to the cell surface in tissues is of pivotal importance with regard to the physiology of local RAS at the tissue level in organs, since it provides a mechanism to generate ANG II locally in excess of the ANG II that is produced in plasma. Previously, it was suggested that binding of prorenin and/or renin is responsible for uptake into tissues (5). It was postulated that this mechanism (which is known also for other enzymes) rather than de novo renin biosynthesis at obscure local sites is responsible for local actions of ANG II. Our understanding of the potential role of prorenin and renin binding has been significantly expanded by the characterization of several proteins capable of binding prorenin and/or renin (815, 488, 490).

The mannose-6-phosphate receptor (M6P) has been shown to be involved in renin and prorenin uptake into cells (132, 602, 733, 737). The cation-independent M6P is directly coupled to G proteins (225, 603) and binds not only prorenin and renin but is in general involved in transport of proteins containing M6P residues; it leads also to insulin-like growth factor II internalization and inactivation (225). M6P was shown to bind renin and prorenin on neonatal rat cardiac myocytes (601) and on human endothelial cells (5). Only glycosylated prorenin and renin are bound by this receptor, and binding is followed by internalization and activation by proteolytic cleavage and subsequent immediate degradation of renin as demonstrated on cardiac cells (488, 601). Particularly studies in endothelial cells indicated that uptake of prorenin by M6P represents a clearance mechanism (733). The role as a clearance receptor on cardiac myocytes is also supported by the finding that intracellular ANG generation could not be demonstrated following (pro)renin uptake in these cells (315, 603). In addition, Peters et al. (542) provided evidence for the existence of a prorenin
binding protein on cardiac cells that is different from the M6P receptor. The functional significance of prorenin internalization in cardiomyocytes was shown in vitro and in vivo, supporting the concept of an intracrine RAS in these cells (488, 542).

It has also been suggested that proteins interacting with renin could act as renin inhibitors in vivo, such as a renin binding protein (RnBP) which was isolated from the porcine kidney (698) and identified in porcine, rat, and human tissues (693, 697, 698). Originally, RnBP was identified as a protein in the kidney that was capable of binding renin in renal homogenates giving rise to a complex designated high-molecular-weight renin (698). Subsequently, RnBP was shown to be identical to the N-acetylglucosamine 2-epimerase (437, 699). The gene encoding RnBP has been mapped on chromosome X (697), and chromosomal fine-mapping in the rat indicated that this locus does not fall within a blood pressure quantitative trait locus previously identified in rat models of hypertension (812). Moreover, knock-out of the gene encoding for this protein in mice did not show any effects on RAS activity or blood pressure (621), suggesting that RnBP is of little functional significance in this context.

In contrast to these negative studies, a specific human renin receptor has been recently identified by expression cloning (490). The complementary DNA of this renin receptor encodes a 350-amino acid protein with a single transmembrane domain and no homology to any known membrane protein (490). High expression levels are detected in the heart, brain, and placenta at the mRNA level, while lower levels are observed in the kidney and liver (488, 490).

This 45-kDa membrane protein binds both prorenin and renin and shows a dual function (488). First, binding of prorenin activates cellular effects that are independent from ANG II generation by activating the mitogen-activated protein (MAP) kinases p(42)/p(44) and extracellular signal-regulated kinases (ERK) 1/2 (488, 490). Second, this receptor acts as a cofactor by increasing the efficiency of prorenin activation in vivo and their physiological role are still not fully characterized (689). Moreover, studies using specific antibodies designed from the tertiary structure of prorenin have shown that there is an essential region in the NH2-terminal region of prorenin, which is responsible for the nonproteolytic form of activation (689). Suzuki et al. (689) have demonstrated two key segments in this region termed the “gate” and “handle” regions. Subsequent studies (296) have further shown that specific binding proteins, interacting with the “handle” region, are responsible for the nonproteolytic activation of prorenin through the induction of a conformational change. These findings shed new light on earlier observations of an independent functional role of prorenin, suggesting that prorenin is a useful marker of diabetic microvascular complications (143, 423, 481) and Wilms’ tumor (379).

In the kidney it has been suggested that prorenin uptake and intrarenal activation of the kidney RAS is responsible for inducing renal damage and microvascular changes (296). Interestingly, an earlier transgenic study has come to similar conclusions using a reverse approach (740). Overexpression of prorenin, subjected to site-directed mutagenesis at its proteolytic cleavage site in transgenic rats, has led to high prorenin levels in the plasma, but also to a severe renal phenotype characterized by severe nephrosclerosis in the absence of elevated blood pressure (740). Now that the mechanisms of nonproteolytic activation of prorenin appear to be deciphered, this could finally solve the controversy of elevated prorenin levels and cardiovascular phenotypes not only in the kidney but also in other organs such as the heart (542). The proposed mechanism could be the basis for the understanding of these phenomena and provide new insights into the function of intracellular RAS and their independence from the regulation of active renin in the plasma. The identification of this receptor could play an important role for the understanding of cellular effects of prorenin/renin regulation of cell-specific ANG II formation. It is likely that this discovery could change our view
of tissue RAS function by defining an independent functional role for prorenin and renin that are independent from the effects of ANG II (and other peptides) generated within the classical RAS cascade. Yet, the function, relevance, and tissue specificity of the renin receptor and other potential pro(renin) binding mechanisms are still poorly defined, and future research has to investigate whether these sites function alone, together, or in combination with other mechanisms at the tissue level (87).

2. ANG-(1—7)

Alternative cleavage products of ANG I have been suggested as functional peptides in the RAS (200, 201, 595, 596). The most extensively studied of these is ANG-(1—7), which is generated by the action of several ACE-independent enzymes from ANG I (200). Some of these enzymes such as neprylisin are also involved in the metabolism of atrial natriuretic factors and bradykinin, and ACE has been shown to be active in metabolism and breakdown of ANG-(1—7), suggesting a complex interaction between different cardiovascular peptide systems (769). ANG-(1—7) has multiple actions that are mostly counteracting those described for ANG II (201, 310, 420, 596).

3. ACE and ACE2

Recently, a novel functional role of ACE involving outside-in signaling has been identified by Fleming and co-workers (208, 353–355). They demonstrated that ACE inhibitor binding to ACE activates ACE-associated casein kinase 2 (CK2)-mediated phosphorylation of ACE Ser-1270 (355). Depending on initial phosphorylation, ACE-kinase 2 (CK2)-mediated phosphorylation of ACE Ser-1270 (355). Depending on initial phosphorylation, ACE-kinase 2 (CK2)-mediated phosphorylation of ACE Ser-1270 (355). Depending on initial phosphorylation, ACE-kinase 2 (CK2)-mediated phosphorylation of ACE Ser-1270 (355). Depending on initial phosphorylation, ACE-kinase 2 (CK2)-mediated phosphorylation of ACE Ser-1270 (355).

The expression of ACE2 is (in comparison with ACE) relatively restricted to cardiac blood vessels and tubular epithelia of the kidneys, which together with its differential enzyme activity might suggest a distinctive physiological function blood pressure and volume regulation (128, 713). A recent study (119) has mapped ACE2 to a region on the X chromosome that is thought to be involved in the genetic modulation of hypertension (271, 794, 812). However, a potential role of ACE2 for genetic hypertension appears questionable since the reported expression analysis of ACE2 in hypertensive rat strains (119) was at variance with the allelic effects at the blood pressure loci observed in mapping analysis with these strains (271, 794, 812). Crackower et al. (119) performed a number of knock-out studies in mice and Drosophila, and while there were only modest effects on blood pressure in mice, the genetic manipulation resulted in severe changes in cardiac contractility, combined with increases of cardiac ANG II levels and the induction of gene pathways mediating the response to hypoxia (119). Therefore, it has been suggested that ACE2 is important as a regulator of heart function and development. Further expression studies of ACE2 on the mRNA and protein level extended the spectrum of organs in which this enzyme is expressed (251, 251, 257) and particularly demonstrated that ACE2 is abundantly present in humans in the epithelia of the lung and small intestine (251). However, taken together, no clear picture of the tissue distribution of ACE2 expression and the physiological role assigned to ACE2 has been yet obtained. Interestingly, work in cell lines suggested that ACE2 is the functional receptor for coronavirus associated with the acute respiratory syndrome, i.e., SARS-CoV (408, 789). The functional role for ACE2 for SARS-CoV replication in vivo was subsequently confirmed in mice (364). Additional studies could demonstrate that ACE2 protects against lung injury caused by SARS-CoV and other agents (301, 364).
4. N-acetyl-Ser-Asp-Lys-Pro

It is well-known that ACE is acting on several substrates such as ANG I and bradykinin. One of these, N-acetyl-Ser-Asp-Lys-Pro (Ac-SDKP), is a hematopoietic factor that is a natural substrate for the NH2-terminal domain of ACE (573). The breakdown of Ac-SDKP can be blocked by ACE inhibitor treatment resulting in an increase of its plasma levels, and it has been suggested that measurement of Ac-SDKP could be a marker for the clinical efficiency of ACE inhibition (25). In the hematopoietic system, Ac-SDKP acts on the cell cycle and prevents the activation of pluripotent stem cells (26), and levels of Ac-SDKP have recently been associated with anemia in heart failure patients treated with ACE inhibitors (734). Although its relevance for cardiovascular regulation still remains unclear, accumulating data indeed support a functional role of Ac-SDKP (554, 555, 569, 761, 800). These functions might include the stimulation of angiogenesis (761) and particularly antifibrotic effects that could point to possible effects in repair and remodeling processes in the cardiovascular system (554, 555, 569, 761, 800) and kidney (88, 646, 744).

5. Chymase

An ANG II-forming serine protease termed human heart chymase has been postulated as an activator in an alternative pathway of ANG II formation in the heart (728, 730). It is not affected by ACE inhibition and has been suggested as relevant for alternative pathways of ANG II generation (728, 730, 781). Although several other alternative enzymes involved in ANG II formation had been described previously such as cathepsins and tonin, chymase deserves special attention due to its high substrate specificity. The enzyme is also expressed in the vascular wall, where it has been suggested as a possible player in ANG II-mediated arteriosclerosis (20). Although its ultimate relevance for the cardiac RAS remains to be determined, since its cellular localization is largely restricted to mast cells (418, 728), experimental studies with selective chymase inhibitors in animal models of heart diseases have thus far generated promising results (167, 700).

6. Angiotensin receptors

The actions of ANG II are mediated predominantly by two seven transmembrane domain receptors termed AT1 and AT2 showing a complex pattern of regulation and function (142, 306, 336, 462, 604, 639, 681, 712, 724, 725, 785). In rat and mouse, two AT1 subtypes have been cloned and characterized; they are termed AT1A and AT1B (302). The AT1 and AT2 subtypes show similar properties of ANG II binding but different genomic structure and localization as well as tissue-specific expression and regulation (142). Whereas most of the well-known actions of ANG II such as vasoconstriction and aldosterone release are mediated by the AT1 receptor, the AT2 receptor has been considered to be more of an enigma (403, 724). It appears to play an important functional role in prenatal development, and in the adult, AT2-mediated actions have been shown to counteract AT1 effects such as cell proliferation in vitro (681) and in vivo (462). Increasing evidence supports a role of AT2 particularly in the regulation of growth, differentiation, and regeneration of neuronal tissue (676). The existence of an additional ANG receptor termed AT4 has been postulated, which is interacting with a truncated ANG peptide, ANG IV or ANG-(3-8) (66, 89, 691, 785). Thus the AT4 receptor was originally defined as the specific, high-affinity binding site for the hexapeptide ANG IV. Subsequently, the peptide LVV-hemorphin 7 was also demonstrated to be a bioactive ligand of the AT4 receptor (453). The AT4 binding site has been found in heart, vascular smooth muscle, kidney, colon, adrenal gland, prostate, and many brain regions in processing sensory and motor function (66, 89, 255, 691, 785).

Two additional novel mechanisms of ANG receptor binding leading to contrasting effects have been recently demonstrated (1, 140, 141, 756). First, AbdAlla et al. (1) reported that the AT2 receptor can directly bind to the AT1 receptor and thereby antagonizes the function of AT1. It was shown that heterodimerization between both receptors led to inhibition of AT1 signaling that was independent of AT2 receptor activation (1). Second, activation of AT1 receptor by autoantibodies is involved in tumorigenesis. It has been suggested that Mas is a functional ANG receptor originally described as a factor involved in tumorigenesis. It has been suggested that Mas is a functional ANG receptor (311), a hypothesis which has been challenged since binding of ANG II to cells expressing Mas could not be shown, suggesting that it is only indirectly involved in ANG II signal transduction. Experiments on Mas knockout mice have indeed shown a functional interaction between Mas and the AT1 receptor (750). This interaction may be attributable to heteroagomeration between Mas and the AT1 receptor and leading to inhibition of ANG II effects mediated by AT1 (359). It remains to be determined, however, whether the proposed effects mediated by ANG-(1-7) or other ANG pep-
tides via Mas could indeed be of functional relevance in vivo (597).

II. LOCALIZATION AND FUNCTIONAL ASPECTS

Whereas many previous studies on localization and function of tissue or local RAS have focused on their pathophysiological relevance, many new aspects of a physiological role for these systems have been uncovered. Cloning of all relevant RAS genes as well as the establishment of transgenic and knock-out models have enhanced this knowledge significantly. It appears likely that local and systemic actions of the RAS have to be integrated in a concerted action of ANG-mediated effects. In addition, an independent function of local RAS (for example, in the brain where the RAS components are also expressed in regions inside the blood-brain barrier) has been postulated. The most significant contribution of the locally acting systems is their function at the cellular level. In this context, paracrine and autocrine effects appear of particular importance, which mediate cell specific effects on cell growth, proliferation, and metabolism. There have also been some suggestions of intracellular or intracrine RAS actions (175) mediated by ANG binding in the cell nucleus (704). Nevertheless, this intracellular concept of ANG II synthesis and function awaits final confirmation.

The purpose of this review is to integrate aspects of localization of local RAS components with function and will focus predominantly on the physiological rather than pathophysiological implications of these findings. In some instances, pathophysiological concepts will be used to enhance understanding of their physiological basis. We do not discuss the RAS in kidney and adrenal gland as these have been extensively reviewed elsewhere (28, 59, 76, 259, 318, 343, 373, 376, 401, 425, 450, 473, 627, 652), and we rather focus on local RAS in other organs that are not typically associated with ANG formation.

A. Heart

The existence and function of a specific cardiac RAS has been a matter of debate since it has been difficult to differentiate the effects of intracardiac ANG II generation from actions by plasma-borne ANG II. Nevertheless, it has become quite clear that cardiac actions of drugs inhibiting ANG II actions such as ACE inhibitors and ANG II receptor blockers are in part explained by local effects at the cellular level, for example, on cardiac remodeling. The predominant physiological role of the cardiac RAS appears to be the maintenance of an appropriate cellular milieu balancing stimuli inducing and inhibiting cell growth and proliferation as well as mediating adaptive responses to myocardial stress, for example, after myocyte stretch. A schematic representation of cardiac ANG pathways is shown in Figure 1.

1. Renin

The existence and relevance of cardiac renin expression has been a matter of controversial debates, although some investigators have been able to detect renin mRNA in the heart by Northern blotting (174), solution hybridization assays (531), and RT-PCR (532) in various species. Renin mRNA expression in all of these studies, however,
was rather low, and very high amounts of mRNA or total RNA had to be used to bring the renin signal to the level of detection. Other investigators challenged these findings and were unable to find proof for local mRNA expression in the literature (751), claiming that the measurement of cardiac renin mRNA was based on contamination problems and artifacts. This was supported by findings that cultured cardiomyocytes or fibroblasts did not synthesize renin. Ultimate proof of the presence of local renin synthesis in the heart was to be expected by transgenic overexpression using the native renin promoter. Transgenic mice carrying a genomic human renin construct showed no cardiac renin expression (798), whereas transgenic rats carrying a genomic construct of the mouse Ren-2 gene under control of its own promoter expressed high levels of renin mRNA in the heart (549). This suggests that at least in some species, the heart is a site of extrarenal renin production. Other authors have suggested that, whereas renin is not synthesized in the heart under physiological conditions, renin gene expression may be turned on in pathophysiological situations (147). It is interesting to note in this context that an additional truncated renin mRNA has been described in heart tissue which lacks the prefragment of preprorenin and results in the formation of a truncated prorenin protein termed exon 1A renin (103, 541). The initial characterization of this isoform in rat adrenocortical cells revealed that this alternative transcript encodes for a truncated prorenin that is imported into mitochondria (104). Subsequent studies demonstrated the expression of this truncated isoform in the rat heart and suggested that only this alternative renin transcript but not the full-length isoform is expressed in the rat heart (103, 541, 542). This conclusion was based on studies in which renin expression was analyzed by a sensitive nested RT-PCR method allowing the differentiation between the full-length transcript coding for preprorenin and the transcript coding for the truncated intracellular isoform (103, 541, 542). While the latter was detected in various rat tissues in parallel with the full-length mRNA, the exon 1A renin was the only transcript of the renin gene expressed in the heart (103, 541, 542). Moreover, it was shown that in disease states such as cardiac hypertrophy or myocardial infarction, there is no expression of the mRNA coding for the full-length preprorenin but exclusively the exon 1A renin transcript is increased (103, 542). The recent findings on the differential regulation of preprorenin, i.e., the classical full-length renin, and exon 1A truncated renin in the heart might explain some of the previous controversies and discrepancies in the literature regarding renin expression in the heart, since investigators were unable to differentiate between the two isoforms before the identification of exon 1A renin (541).

Less controversial is the evidence for the presence of renin protein in the heart attributed to uptake from the circulation (134) either due to nonspecific uptake (diffusion) into the cellular interstitium (144, 315, 542) or through the actions of specific functional binding sites or receptor for prorenin and renin (87, 488, 490, 541). The M6P receptor has been shown to bind prorenin and renin cells on cardiomyocytes (601, 603). Hypertrophy of isolated neonatal cardiomyocytes in culture and increase in protein synthesis were only detected during coinubation of prorenin with angiotensinogen, while prorenin binding alone had no effect (603). The effects of prorenin plus angiotensinogen were comparable to those of 100 nM ANG II, although the ANG II levels in the medium during exposure of the cells to prorenin plus angiotensinogen were <1 nM. This suggests that cardiac ANG II generation by circulating renin occurs predominantly on the cell surface (315, 603). In addition, the newly identified prorenin and renin receptor that is capable of activating signal transduction via MAP and ERK kinases independently from ANG II generation shows high expression levels in the heart (488, 490). Taken together, these findings support the concept that the physiological role of the tissue RAS in the heart depends on the assembly of prorenin and renin binding receptors including M6P and ANG receptors (488, 603). This favorable microenvironment would allow maximal efficiency of local ANG II generation, i.e., immediate binding of ANG II to its receptors with minimal loss into the extracellular space (315, 488, 603).

A recent study could show that unglycosylated renin is rapidly taken up by cardiomyocytes by a mechanism that is independent from the M6P receptor and that transgenic rats with overexpression of the mouse Ren-2 gene (which have high prorenin levels in the plasma) exhibit strongly enhanced intracellular levels of unglycosylated renin in their hearts (542).

2. ACE

The existence of local ACE production in the heart is, in contrast to the renin, no matter of controversy. Cardiac ACE mRNA can be easily detected by a number of methods in rat (277, 362, 626) and human hearts (532). ACE activity is also readily detectable, for example, by autoradiography (796) or enzymatic assay (278, 362, 726). Immunohistochemistry has been used to localize the predominant source of ACE expression in cardiac blood vessels and the endocardium (190), whereas mRNA studies on cultured cardiac cells also found ACE expression in cardiomyocytes (536). These studies have been confirmed by expression studies showing that ACE is present in viable human cardiomyocytes after myocardial infarction (284). ACE2 expression has also been demonstrated in the heart in both animals (119, 713) and humans (67, 238).
3. Chymase

Human heart chymase activates ANG I to ANG II but is not inhibited by ACE inhibitors and could act as an activator for alternative pathways of ANG II formation. Using whole heart homogenates, Urrata et al. (729) described that up to 80% of ANG II forming activity in the heart was due to chymase, while only 11% was based on ACE activity, suggesting an increased importance of the chymase pathway in the human heart. When comparing studies in humans and animals, it is important to consider the important species differences in the pathways of intracardiac ANG II generation (30). In this regard, chymase predominates over ACE activity in human heart, accounting for considerably higher total ANG II formation in human heart compared with dog, rat, rabbit, and mouse hearts (30). The ultimate functional importance of a chymase-dependent pathway of ANG II formation in the heart remains questionable due to a number of factors: 1) clinically, ACE inhibitors are extremely efficient in the treatment of cardiac disease; 2) under experimental conditions, most of the ANG II generated by intact cardiac blood vessels can be blocked by ACE inhibitors; and 3) the expression of human heart chymase is highly compartmentalized and mostly restricted to mast cells (728). Nevertheless, ANG II-generating pathways in the heart that are independent from ACE might be particularly important in disease states such as cardiac hypertrophy (407) and heart failure (781).

4. Angiotensinogen, ANG I, and ANG II

The detection of angiotensinogen mRNA in the heart has been described for mouse (174), rat (268, 417), dog (382), and human (532). Although the cardiac mRNA levels of angiotensinogen are more readily detectable than those of renin, they are low compared with those found in liver, the major source of angiotensinogen production (174). Arguments against local mRNA synthesis of cardiac angiotensinogen stem from experiments in isolated perfused rat hearts where there was no endogenous angiotensinogen release detectable (144). These authors concluded that the major percentage of cardiac angiotensinogen is due to plasma uptake and presented evidence that the protein is rapidly taken up into the cardiac interstitium when added to the perfusate.

ANG peptides have been detected in the heart (132, 416) at concentrations higher than those found in the plasma compartment. Although this could be seen as an indicator of cardiac synthesis, the issue of cardiac uptake and local storage of ANG II should be considered. Alternatively, renin and angiotensinogen taken up from the circulation could be interacting with local ACE to lead to intracardiac ANG II formation. To address this question, van Kats et al. (736) used infusions of radiolabeled ANG I and ANG II peptides in pigs and measured plasma and tissue levels of endogenous as well as the radiolabeled peptides. The results of this study indicated that >90% of cardiac ANG I is synthesized locally in the heart and that >75% of cardiac ANG II is synthesized locally, most of it using local ANG I generation as a basis. These findings clearly show the local synthesis of ANG peptides in the heart as a relevant mechanism and point out that the concept of a cardiac RAS is not dependent on the local synthesis of angiotensinogen and renin.

5. Angiotensin receptors

Both the AT1 and the AT2 receptors are expressed in the heart where they appear to be localized on cardiomyocytes (28, 55, 577, 590, 729). On cardiac fibroblasts, the receptor population appears to be dependent from the presence or absence of cardiac disease. Normal fibroblasts express AT1 only but can recruit the AT2 receptor under certain pathological conditions (118, 510, 635). The function of the two ANG receptors in the heart has been seen in perspective of a “Yang-Yang” principle, meaning that the AT1 is a stimulator of hypertrophy and proliferation of cardiac cells, whereas AT2 is mediating the opposite effects (773). Transgenic and knock-out studies in mice, however, have not supported this concept in the heart, since knockout of the AT2 receptor in mice has suggested that the receptor is also needed for the mediation of hypertrophic stimuli (705). Moreover, conflicting experimental data obtained more recently in animal studies using either selective AT2 antagonists or genetically modified mice have raised some concern regarding the beneficial role of AT2 stimulation in both the heart and vasculature in disease states (403).

6. Function

A) Inotropic effects. Koch-Weser (351) first suggested that ANG II acts as an inotropic agent (351) but only at “supraphysiological” concentrations. The effect could at least in part be indirect by ANG II acting, for example, on the sympathetic nervous system (352). Nevertheless, direct effects by ANG II have been verified (148), which are thought to be mediated by intracellular calcium influx and changes of the plateau phase of the cardiac action potential. In vitro studies in human preparations carried out under physiological conditions in right atrial and right and left ventricular myocardial preparations of patients with a variety of cardiac diseases suggested that ANG II exerts positive inotropic effects only in atrial preparations (286). However, transgenic overexpression of the human AT1 receptor on cardiac myocytes in a transgenic rat model has supported the early findings since transgenic AT1 upregulation led to an enhanced intracellular calcium response after ANG II stimulation (280).

B) Hypertrophic effects. ANG II mediates myocyte hypertrophy due to activation of the AT1 receptor as an
adaptive response to increased myocardial stress. While hypertrophy of cardiomyocytes acts initially as a compensatory mechanism to preserve cardiac function, it becomes a major risk factor for congestive heart failure and sudden cardiac death and overall mortality (172). In vitro studies have demonstrated this effect in cultured cardiomyocytes (738), and it has been suggested that this effect is secondary to the release of other growth factors such as endothelin-1 and transforming growth factor (TGF)-β (239). Left ventricular hypertrophy due to enhanced ANG II production in the heart has also been described in the transgenic rat models with overexpression of RAS components such as mouse renin (549), the human AT1 receptor (279, 280), human ACE (555, 711), and double transgenic rats expressing human renin and human angiotensinogen (470). In some of these models it has been clearly shown that the ANG II effects occur independently from its effects on blood pressure, suggesting a functional role of the local cardiac RAS in mediating these changes. Hypertrophic changes induced by ANG II are mediated by several distinct intracellular pathways such as the activation of tyrosine kinase and RhoA cascades which include activation of MAP kinase and JAK/STAT pathways (142, 435, 613, 725). An important maladaptation in left ventricular hypertrophy relates to diastolic dysfunction that results from functional changes such as impaired diastolic calcium handling and/or structural changes such as cardiac fibrosis (101, 586, 629). Studies in rats with experimental left ventricular hypertrophy indicated that locally generated ANG II may disturb relaxation, i.e., diastolic function, of the heart (629). This notion was supported by subsequent studies in transgenic rats with activated tissue RAS showing significant impairment of diastolic relaxation (585, 586). Moreover, diastolic function could be restored by treatment with a non-blood pressure-lowering dose of an AT1 receptor antagonist (586). Functional analysis of left ventricular dysfunction in this setting indicated that the impairment of diastolic dysfunction was attributable to impaired diastolic sarcoplasmic reticulum calcium pump (SERCA2) activity (586). A similar effect could also be induced in the same rat model by treatment with a selective endothelin A receptor antagonist, demonstrating the activation of the endothelin system in rats with activated tissue RAS and its functional consequence in the heart (585). Chronically, activation of the cardiac RAS may not only lead to cardiac hypertrophy and diastolic function but also to progressive systolic dysfunction, cardiac enlargement, and heart failure. The independent role of cardiac RAS activation for these consequences has been recently demonstrated in transgenic TG1306/1R mice that develop ANG II-mediated cardiac hypertrophy in absence of elevated blood pressure (168). A long-term follow up study in these mice demonstrated that transgenic animals develop dilated cardiomyopathy with aging and exhibit a significant increase in mortality compared with wild-type mice. Cardiac hypertrophy in transgenic mice is also associated with SERCA2 activity and reduced Ca2+ transport. Moreover, systolic function was also impaired as evidenced by impaired contractility in isolated cardiomyocytes (168). Equivocal results have been obtained with regard to the role of the AT2 receptor in cardiac hypertrophy (403). While the AT2 receptor has been initially associated with antihypertrophic effects (403), some studies in AT2-deficient mice indicated that AT2 has a significant effect on cardiac hypertrophy induced by aortic banding (635) or ANG II infusion (297).

C) Mechanical stretch. Several lines of evidence indicate that ANG II pathways can be defined as a "rapid response system" for mechanical stretch in the heart, which may be involved in the mediation of cardiac hypertrophy (100). Stretch can induce ANG II release into the media of cultured cardiomyocytes in vitro (587) and in vivo (391), and virtually the expression of all gene transcripts of the RAS can be stimulated by stretch (432). Overload of the left ventricle, which represents a situation associated with chronic stretch of cardiomyocytes, results in a similar activation of the cardiac RAS (29, 626). The intracellular pathways activated by the induction of the cardiac RAS and local ANG II production in cultured neonatal cardiomyocytes are blocked by AT1 receptor antagonism (357). The signaling is mediated by p53 as well as by the JAK/STAT pathway (391). Whereas initially it was thought that these effects are mediated entirely through the AT1 receptor, recent AT2 knock-out studies in mice have revealed that the AT2 receptor is also involved in mediating these changes (635).

D) Remodeling. Proliferative stimuli by cardiac ANG II are probably most relevant for the fibroblast portion of cardiac cell population as has been shown by Schelling and Ganten (606, 608). Similar mechanisms have been described during cardiac remodeling where fibroblast proliferation has been shown as a cellular indicator of pathological changes. Local ACE formation appears to play an important role in this process, since previous studies in a rat heart failure model induced by experimental myocardial infarction (which goes along with increased fibrosis) have demonstrated an activation of cardiac ACE activity and mRNA, whereas plasma ACE activity was not changed (277).

In rat hearts, Sun and Weber (687) have shown that at weeks 1 and 4 after myocardial infarction myofibroblasts were the predominant cell expressing high-density ANG II receptors at this site, while fibroblasts, macrophages, and vessels demonstrated low-density ANG II receptor binding. After myocardial infarction in rats treated with the AT1 antagonist losartan, a significant reduction in collagen volume fraction at remote sites of the infarction was found compared with untreated animals (139).

Since either AT1 blockade or ACE inhibition is not associated with any normalization of elevated collagen
mRNA after rat myocardial infarction, Dixon et al. (165) suggested that the reduction of cardiac fibrosis mediated by ACE inhibition and losartan treatment may reside at the posttranslational level in cardiac collagen metabolism. The RAS has apparently multiple targets involved in cardiac remodeling. Tan et al. (702) measured the cardiotoxic effects of ANG II in rats. The authors have shown that pathophysiological levels of endogenous as well as nonhypertensive low doses of exogenous ANG II produced multifocal antimony labeling of cardiac myocytes and myocytolysis, increased DNA synthesis rate and fibroblast proliferation. Both myocyte injury and fibroblast proliferation were prevented with captopril (702).

The mechanisms leading to ANG II-induced fibrosis are thought to be at least partially mediated through growth factor pathways induced by AT1 receptor activation (548, 686). In this context, TGF-β has been implicated as a candidate. Studies on transgenic rats expressing the mouse Ren-2 gene have shown that inhibition of TGF-β synthesis by the specific growth factor inhibitor tranilast did not affect blood pressure but resulted in a significant alleviation of interstitial cardiac fibrosis seen in this model, which was also associated with longer survival of treated transgenic animals (548).

Another mediator, osteopontin, which is involved in the vascular smooth muscle cell remodeling process, is increased at mRNA and protein levels after addition of ANG II to rat cardiac fibroblasts (24, 506). This effect is blocked by the AT1 receptor blocker losartan. This suggests that osteopontin is a potentially important mediator of ANG II regulation of cardiac fibroblast behavior in the cardiac remodeling process. Indeed, osteopontin mRNA is elevated in transgenic rats, the mouse Ren-2 gene already in the prehypertensive phase, which suggests that it contributes directly to the contractile dysfunction seen in this model and is blood pressure independent (584).

Yet another mediator in the inhibition of cardiac fibroblast proliferation appears to be the recently described alternative substrate of ACE, AC-SDKP, a hematopoietic stem cell regulator, is hydrolyzed by the NH2-terminal active site of ACE. It has been demonstrated that the administration of ACE inhibitors stimulates AC-SDKP plasma levels over fivefold (25), which could be a plasma marker for efficient ACE inhibition. Recently, it has also been demonstrated that AC-SDKP inhibits also fibroblast proliferation in a dose-dependent manner (554), suggesting that the alternative substrate could be the mediator of antiproliferative effects on fibroblasts in cardiac remodeling seen after ACE inhibition. This fascinating hypothesis, however, awaits final confirmation.

In addition to the structural abnormalities related to activation of the cardiac RAS, increased activity of the system has also been linked to changes in the electrical physiology that lead to arrhythmias both in the ventricle and atria (146, 266). Indeed, in human patients undergoing heart surgery, patients with a history of paroxysmal or persistent atrial fibrillation showed increased interstitial fibrosis and threefold higher ACE tissue levels compared with patients in sinus rhythm (233), while the densities for the AT1 receptor was decreased and increased for AT2 receptors (231). Most importantly, evidence obtained from recent pharmacological intervention studies has pointed to a new concept in which inhibition of the RAS by ACE inhibitors or AT1 antagonists may induce specific benefits in patients with atrial fibrillation (232, 265, 649).

E) Apoptosis. Whereas earlier studies carried out in PC-12 cells (a rat pheochromocytoma cell line) have suggested that programmed cell death is mediated by the AT2 receptor (797), it is generally accepted that apoptosis of cardiac myocytes is mediated via the AT1 receptor (99). The process is thought to be involved in cardiac remodeling, for example, after myocardial infarction (19), hypertensive cardiomyopathy (163), and diabetic cardiomyopathy (206). It can be effectively blocked by AT1 antagonists (160), which suggests that the beneficial effects of RAS blockade in heart failure could be due in part to this intracardiac mechanism.

B. Vasculature

The vascular wall is the effector organ for the hormonal or plasma RAS where AT1 receptors localized on vascular smooth muscle cells mediate vasoconstriction. The concept of a vascular RAS was generated when it became evident that ANG II can differentially affect growth properties of vascular cells and that RAS components can be formed intracellularly in the vasculature.

1. Renin

Whereas some studies have not found renin activity in blood vessels (210), others have found renin mRNA expression in conductance and resistance vessels of human (532) and rat (594). Nevertheless, renin mRNA has been difficult to detect due to the small sample size which very often required pooling of samples and the use of more sensitive assays such as RNase protection assays as well as RT-PCR. This has led to a similar controversy as that concerning renin in the heart, and it has been proposed that local renin synthesis is negligible if at all present in the vasculature (751). Although this may be true under physiological circumstances, it is possible that local renin production may be turned on in disease states. In this context, vascular renin induction has been demonstrated in the rat neointima model (309).

In addition to local synthesis, renin uptake via unspecified binding sites on endothelial cells or specific prorenin/renin receptors (87, 315, 488, 490) has been suggested as a relevant mechanism. The M6P receptor was shown to bind renin and prorenin on human endothelial
cells (5), and studies in endothelial cells indicated that uptake of prorenin by M6P may represent a clearance mechanism for prorenin (733). This could in fact provide a protective mechanism during activation of the RAS either systemically or at the tissue level. The potential importance of this mechanisms was shown by generation of a transgenic rat model that directed high prorenin expression and release into the plasma from the liver (740). This led to dramatic increases in plasma prorenin that were up to 400-fold. Interestingly, these animals did not have higher blood pressures than controls, yet developed severe vascular lesions, suggesting that prorenin is taken up from the circulation and stimulating the intracellular RAS leading to pathological trophic effects (740). Moreover, in humans, increases in circulating prorenin levels have been associated with disease states such as diabetic microvascular complications (143, 423, 481). It thus appears of interest for further studies to evaluate the functional role of prorenin clearance and elimination in the endothelium by M6P or other mechanisms. These effects have to be balanced against mechanisms such as binding by the prorenin/renin receptor (490) or nonproteolytic activation of prorenin (296, 689) that would lead to the activation of the RAS (87, 315, 488, 490).

2. ACE

In the vascular wall, ACE is readily detectable, where it is localized predominantly on the surface of endothelial cells (190, 811). There are controversial data regarding the distribution of ACE expression in different layers of vascular wall. Wilson et al. (776) found a predominant labeling in the endothelium and adventitia. This finding was confirmed by Rogerson et al. (578) in human, dog, rabbit, and sheep arteries. Arnal et al. (22) have shown high ACE mRNA and protein expression as well as immunoreactivity in the media of rat aorta where the expression level is almost as high as in the endothelium, while expression is low in the adventitia. Several reports have indicated that vascular smooth muscle cells, which do not appear to express ACE, can do so in certain pathophysiological situations such as neointima formation (197). Low amounts of ACE have also been detected in the adventitia of blood vessels (578).

ACE2 mRNA expression is ubiquitously found in arterial and venous endothelial cells and arterial smooth muscle cells in all organs studied by Hamming et al. (251). An important role for ACE and ACE2 in the pulmonary vasculature and epithelial cells during injury and/or SARS virus infection has been recently supported (301, 364, 491). However, further studies are required to delineate the cell types responsible for RAS component expression in the lung and to identify the physiological role of the pulmonary RAS (436). This will also provide a better understanding of the activated RAS, and particularly the potential protective effect of ACE2 in lung disease (436, 491).

3. Angiotensinogen, ANG I, and ANG II

The substrate of the RAS cascade has been detected in blood vessels at the mRNA level (74, 268). In situ hybridization studies showed that it is abundantly expressed in periventricular fat cells (73, 84). This led to the suggestion that angiotensinogen is secreted by these cells and diffuses through the vascular wall where it gets in contact with vascular renin.

4. Angiotensin receptors

Both AT$_1$ and AT$_2$ receptors were identified in the vasculature (54, 475). Initially the function of these receptors was investigated on cultured primary vascular cells. Vascular smooth muscle cells in culture express only the AT$_1$ receptor, whereas cultured endothelial cells expressed both AT$_1$ and AT$_2$ (681). ANG II stimulated growth of vascular smooth muscle cells, while the peptide inhibited the growth of quiescent coronary endothelial cells in response to stimulation by basic fibroblast growth factor (681). In the presence of an AT$_2$ receptor antagonist, this effect was abolished, suggesting a growth-inhibiting role of AT$_2$ (681). Later, this interesting concept was also reproduced in an in vivo experiment of AT$_2$ gene transfer in balloon-injured rat carotid arteries, which led to a reduction of neointima formation, an effect which was neutralized by an AT$_2$ antagonist (461). The distribution of the AT$_2$ receptor in the vascular wall is nevertheless a matter of debate (39). Overall, functional studies in isolated arteries from both animals and humans accumulated a large body of evidence that the endothelium is the most important side for AT$_2$ receptor expression (38, 39, 46, 234, 254, 622). In addition, since AT$_2$ may form heterodimers with AT$_1$ receptors (1), this would require a colocalization of both receptors and thus points to the additional expression of AT$_2$ expression on vascular smooth muscle cells in the vascular wall (39).

5. Function

A) VASCULAR TONE AND ENDOTHELIAL FUNCTION. The tissue RAS contributes to the maintenance of cardiovascular homeostasis by the dual impact on vessel function mediated through the opposing effects of its two receptors. In in vivo studies, i.e., in the whole body situation, it is not possible to clearly dissect between effects mediated by ANG II generated in the plasma and effects attributable to ANG II generated within the vessel wall. Nevertheless, earlier studies documented the potential of ANG II generation within the vasculature. The local generation of ANG II in the vasculature has been demonstrated in isolated perfused rat hindquarters (272). Hilgers et al. (272) reported that ANG I can be converted to ANG II by ~50%
during one pass through a hindlimb. This conversion was abolished by ACE inhibition. Thus these data supported the presence of a functional vascular RAS in which ACE contributed to the local formation of ANG II. In subsequent experiments it was shown that the vascular production of ANG II was endothelium mediated, since the conversion of ANG I to ANG II was abrogated by endothelium denudation (273).

In response to either systemically or locally generated ANG II, the AT1 receptor mediates the contractile response by phospholipase C-dependent mechanisms leading to an increase in intracellular calcium (142, 604). It also acts indirectly, by stimulating the synthesis of other vasoconstrictors such as endothelin-1 (535, 582, 614). In contrast, activation of AT2 receptors results in activation of protein phosphatases, thereby reversing the effects mediated by ANG II binding to AT1 receptors (505). Thus the AT2 receptor appears to mediate mechanisms that are counterregulatory and prevent the occurrence of pathological vascular changes (77, 773). The functional role of the AT2 receptor was primarily seen during fetal development during which AT2 is highly expressed (639, 724). Subsequently, reappearance of AT2 in the adult organism was associated with pathological events that can be viewed as fetal-reprogramming. This, however, does not rule out the possibility that AT2 plays an important role for the regulation of vascular tone and blood pressure under physiological conditions (39). Indeed, a large body of evidence obtained primarily in animals supports a role for AT2 receptor-mediated vasodilation (39, 234, 234, 758). Importantly, however, this vasodilatory role of AT2 has been also clearly demonstrated in human coronary microarteries (38, 39), while the effects in larger human coronary arteries (38) and human forearm resistance vessels (429) were not significant (38, 39). Collectively, the vasodilatory effect mediated by AT2 receptors appears to be predominantly attributable to direct activation of the nitric oxide (NO)-cGMP pathway, while indirect activation of NO via bradykinin and B2 receptors as mediators of AT2-induced vasodilatation is less well established (39). However, recent experimental work in hypertensive rats clearly demonstrates that AT2 receptor-mediated vasodilatation in vivo depends on the blood pressure status (807). In addition to the modulation of vascular tone by ANG II via the NO-cGMP pathway, research carried out during the last decade has clearly shown that the effects of ANG II in the vasculature are mediated at least in part by the modification of the redox milieu of its target cells (253). ANG II has been shown to activate the vascular NAD(P)H oxidase(s) resulting in the production of reactive oxygen species (ROS), namely, superoxide and hydrogen peroxide (71, 240, 253, 457, 565, 637, 714). Consequently, ANG II is capable of increasing NO bioavailability by activation NO-cGMP pathway via AT2 and decreasing NO bioavailability by promoting oxidative stress via the AT1 receptor (71, 253, 623). In this regard, ANG II plays an important role in modulating the balance between NO and ROS in the endothelium and thereby maintaining homeostasis of the vascular wall. Oxidative stress has been shown to play a critical role in the development of endothelial dysfunction and hypertension and atherosclerosis (4, 253, 623). Nevertheless, the relative contribution of redox-independent hemodynamic mechanisms and oxidative stress in maintaining vascular function and their modulation by ANG II via AT1 and AT2 receptors needs further in-depth analysis.

6. Tissue remodeling

In addition to the well-established long-term effects of the RAS on vascular remodeling that are mediated by proliferative effects on vascular smooth muscle cells and fibroblast (9, 75, 223, 227, 607), the production of ROS by NAD(P)H oxidase in response to ANG II has been demonstrated as an important mechanism linking activation of the RAS to events such as inflammation, atherosclerosis, hypertrophy, remodeling, and angiogenesis (71, 253, 623).

The production of ROS by NAD(P)H oxidase in response to ANG II stimulation in endothelial and vascular smooth muscle cells activates signal pathways such as MAP kinases, tyrosine kinases, and transcription factors that lead to these events (71, 240, 253, 623). In the ANG II/AT1-driven processes leading to vascular damage and chronic atherosclerotic by increased production of ROS by ANG II, additional mechanisms such as low-density lipoprotein (LDL) oxidation and uptake, increased LDL-receptor expression (338, 494), increased expression of molecular mediators of inflammation such as NFkB or cell adhesion molecules such as vascular cell adhesion molecule (VCAM)-1 or intercellular adhesion molecule (ICAM)-1 is involved (96, 161, 424, 526). In addition, chemokines and proinflammatory cytokines are activated (96, 424). Moreover, apoptotic changes in response to ANG II in the vascular wall have been described (162). This, in addition to ANG II-induced modulation of extracellular matrix components by matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs), may play a role in vascular remodeling (52, 312) including processes such as the disruption of the normal endothelial layer in early atherosclerosis or plaque rupture in more advanced atherosclerosis (102). Moreover, AT1 and AT2 receptors have been shown to induce opposing effects on vascular smooth muscle cell growth (295). The AT2 receptor may again be considered as a balancing principle that counteracts many of the mechanisms indicated above that are mediated by the AT1 receptor. Percutaneous transluminal coronary angioplasty (PTCA) injury in humans results in upregulation of ACE at sites of active repair (509). After balloon injury of rabbit carotid artery
and after pretreatment with perindopril, there was a reduced neointima formation (316). In contrast, Dale and Blaine (127) have shown that enalaprilat has no effect on neointimal growth or cell proliferation in a vascular organ culture model of rabbit, which may be due to model-specific effects. The blockade of the AT\textsubscript{1} receptor reduced also neointima growth in a manner similar to that of ACE inhibition. The pretreatment with an AT\textsubscript{2} receptor antagonist, CGP 42112A, did not change the neointima-media ratio (127). The direct involvement of the AT\textsubscript{2} receptor in reducing neointima formation, however, has ultimately been shown by direct gene transfer (463). The functional importance of AT\textsubscript{2} has indeed been supported by a study demonstrating that chronic antagonism of AT\textsubscript{2} receptors led to severe vascular changes such as aortic aneurysms and arteriosclerosis (136). The vascular changes in AT\textsubscript{2} knockout mice were less dramatic but pointed in a similar direction, since animals show elevated blood pressure and an increased sensitivity for DOCA-salt hypertension (241). The intracellular mechanisms of these changes have been addressed in vascular smooth muscle cells from AT\textsubscript{2} overexpressing transgenic mice (441). The results of this study suggest that AT\textsubscript{2} activation inhibits JNK and c-Jun expression. However, conflicting results have also been generated during the last decade, questioning the beneficial role of AT\textsubscript{2} stimulation in the vasculature, particularly under pathological conditions (403, 404). Levy et al. (404) demonstrated in normotensive Wistar rats receiving hypertensive doses of ANG II that chronic blockade of AT\textsubscript{2} with the selective antagonist PD123319 had no effect on arterial pressure but antagonized the effect of ANG II on arterial hypertrophy and fibrosis. Moreover, chronic treatment with a selective AT\textsubscript{1} antagonist lowered blood pressure but still led to smooth muscle cell hypertrophy and hyperplasia (404). These data suggest that the detrimental in vivo effects of ANG II on vascular remodeling may be mediated at least in part via AT\textsubscript{2}. In this regard, AT\textsubscript{2} may contribute to vascular adaptation and remodeling by influencing apoptosis in vascular smooth muscle cells (433). However, the effect of AT\textsubscript{2} stimulation on vascular smooth muscle cells may differ between different cell phenotypes (37) and on the coadministration of AT\textsubscript{1} antagonists (706).

7. Angiogenesis

Walsh et al. (757) indicated that ANG II can stimulate angiogenesis, acting via AT\textsubscript{1} receptors within the subcutaneous sponge granuloma model in the rat and that AT\textsubscript{1} and AT\textsubscript{2} receptors and ACE develop sequentially during microvascular maturation. This confirms earlier reports that ANG II stimulates angiogenesis in the chick embryo model (387). These authors have also indicated that an alternative ANG II receptor is mediating these changes (386). It is unclear whether this alternative mechanism is explained by the antiangiogenic actions of ANG-(1—7), which can stimulate NO release by interaction with a non-type 1 or type 2 ANG receptor (431). Studies in AT\textsubscript{2}-deficient mice and wild-type mice using the surgically induced hindlimb ischemia model indicated that AT\textsubscript{2} confers an antiangiogenic effect that is associated with activation of apoptosis (655). This experiment would question the beneficial role of AT\textsubscript{2} stimulation in the context of pharmacological AT\textsubscript{1} blockade in ischemic tissues (403). Interestingly, Ac-SDKP, which is inactivated by ACE, has been shown to promote neovascularization in the cornea and capillary density in the heart (761).

8. Gender difference in cardiovascular RAS

The tissue RAS plays an important role in organs of the reproductive system, and components of the RAS are influenced by gender and sex hormones (207). A large body of evidence has been obtained through research in animals that components of the RAS are significantly influenced by gender effects at the tissue levels (21, 182, 214, 216, 237, 274, 492, 495), while clinical studies in humans have predominantly evaluated gender effects on the circulating, i.e., systemic, RAS (98, 129, 235, 261, 262, 374, 625, 634). Overall, the role of RAS to explain gender-related differences in the cardiovascular system has been addressed by many studies in both animals and humans. The characterization of an estrogen-responsive element in the 5'-flanking region of the angiotensinogen gene has been an important early finding to prove an interaction with sex hormones and the RAS at the molecular level (192, 237). In addition, angiotensinogen expression in the liver is reduced by castration and increased by testosterone treatment in rats (182). While earlier studies suggested that renin activity in plasma is stimulated by estrogens, more recent studies indicated that renin is actually suppressed by estrogens (207, 508, 625). Castration of male rats leads to reduction, while testosterone treatment in ovarietomized female rats increased plasma renin activity (182). Collectively these studies are in line with the observation that renin plasma levels are lower in women compared with men (207, 625). However, the expression of ACE mRNA was significantly lowered in response to estradiol in kidney, lung, and aorta in rats (216). In the heart, ventricular ACE is more abundant in male than female mice at both mRNA and protein levels after reaching sexual maturity and with increasing age (214). Oophorectomy leads only to a slight increase in ACE levels in female mice, whereas ventricular ACE levels are substantially decreased in androgen-deprived males (214). Thus the antithetical changes in ventricular ACE abundance seen in agonadal male and female mice suggest that testosterone as well as estrogen may play a role in regulating ACE expression in the heart (214). In humans, circulating ACE levels appear also to be lower in response to estro-
gens (558, 625, 634). Similarly, AT$_1$ receptor is also downregulated by estrogens while estrogen deficiency leads to upregulation of this receptor (492). In female transgenic rats with activated tissue RAS, it was shown that estrogen treatment is protective against hypertension, possibly by amplifying the vasodilator contributions of ANG-(1–7), while reducing the formation and vasoconstrictor actions of ANG II independently of changes from plasma renin activity (61). In summary, the net effect of estrogens seems to result in a suppression of the RAS both systematically and at the tissue level (207). The role of testosterone or androgens has been less well characterized, although more recently Baltatu et al. (32) demonstrated that treatment with the androgen receptor antagonist flutamide protects against hypertension and end-organ damage not only in male but also in female TGR(mREN2)27 rats.

Despite the evidence that both the activity of the RAS and cardiovascular morbidity and mortality show gender differences, it is not fully uncovered to what extent gender and sex hormones regulate the RAS and thereby mediate effects on the cardiovascular system in men as well as pre- and postmenopausal women during physiological conditions (207). Moreover, further study of this issue appears of interest for several important issues of clinical medicine such as cardiac and vascular remodeling processes in cardiovascular diseases and with ageing as well as the role of hormone replacement therapy (583). In this regard, a more recent study is of interest investigating the variation of the RAS in healthy, premenopausal women during predefined phases of the normal menstrual cycle (98). In this study it was shown that resting blood pressures were lower in the luteal phase of the cycle with high-estrogen phase (days 15–24) compared with the low-estrogen phase of the cycle despite acute increases of renin and aldosterone during the high-estrogen phase (98). In addition, in postmenopausal healthy and normotensive women it was shown that estrogen replacement therapy increases ANG II yet lowers blood pressure, thus suggesting that compensatory mechanisms probably at the tissue level may protect against the systemic activation of the RAS (260).

C. Nervous System

1. Central nervous system

The postulation of an independent brain RAS was one of the first indications of the presence of local ANG formation in tissues. Bickerton and Buckley (50) had already shown in 1961 specific central ANG actions using cross-circulation studies in the dog, and several authors (202, 635) could demonstrate an interaction between ANG II and the autonomous nervous system. These data soon led to the identification of RAS components in the brain.

The existence and functional relevance of the brain RAS is in the meantime fairly established (31, 592). The concept of local ANG synthesis in the brain is shown in Figure 2.

A) RENIN. Renin activity within the brain was first reported by Ganten et al. (220). Although this novel concept has been challenged by some authors (244), the initial finding has been confirmed by studies on the subcellular localization of brain renin in synaptosomes (530) as well as gene expression studies. Renin mRNA was found in the rat and mouse brain (176, 179) albeit at low levels of expression (307, 513, 531, 692). Renin activity is present in many brain regions of the rat and decreases with the age of the animals (611). High expression levels of the recently identified prorenin/renin receptor were also detected in the brain (488, 490). Renin-like activity is high in hypothalamus and pituitary and pineal glands from rat (270), mouse (666), hog (276), as well as rat and dog (219). Renin protein is measurable in rat primary cultures of brain cells and neurons, and a strong staining in glia cells (270) has been observed. Okamura et al. (512) demonstrated renin as well as ANG I and ANG II and ACE in neuroblastoma cells. With the use of double or triple immunogold labeling methods, angiotensinogen, prorenin, and renin were found in lactotrope, gonadotrope, somatotrope, corticotrope, and thyrotrope glandular cell

![FIG. 2. RAS in the brain. Two possible ways of ANG II generation within the CNS are discussed: 1) volume transmission: extracellular generation of angiotensin peptides, acting as neurohormones; and 2) wiring transmission: uptake of angiotensinogen by neurons and formation of peptides within the neuron. ANG II acts as neurotransmitter or cotransmitter (528).](http://physrev.physiology.org/)

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types of the rat anterior pituitary (742). The highest levels were detected in lactotropes and gonadotropes.

B) ACE. ACE mRNA is expressed in choroid plexus, caudate putamen, cerebellum, brain stem, and hippocampus of rat brain (772). ACE mRNA was also found in the pineal gland of rat (33). The localization of ACE protein in the brain was verified by quantitative autoradiography (90, 445, 550, 683), with high levels in the choroid plexus, blood vessels, subfornical organ, and organum vasculosum of the lamina terminalis, with somewhat lower levels in the thalamus, hypothalamus, basal ganglia, and posterior pituitary gland. ACE colocalized with renin in synapticosomal fractions of these brain structures (530). By autoradiography the highest concentrations of ACE were found in the choroid plexus, blood vessels, subfornical organ, vascular organ of the lamina terminalis, area postrema, and inferior olive of the rabbit, and intermediate levels of binding were found throughout the basal ganglia, midbrain, central gray and the superior colliculus, solitary tract nucleus, and dorsal motor nucleus of vagus (579).

C) OTHER ANG II FORMING ENZYMES. Possible enzymes involved in brain ANG formation are tonin (615), cathepsin G (349), tissue plasminogen activator, and chymase (34). Their ultimate functional role in brain ANG formation, however, remains to be determined.

D) ANGIOTENSINOGEN, ANG I, AND ANG II. Angiotensinogen mRNA was described in rat brain by Campbell et al. (72) and Ohkubo et al. (511). Other investigators (427) verified these findings using in situ hybridization and localized angiotensinogen mRNA in rat cells that stained for glial fibrillary acid protein, which is a marker for glial cells. Baltatu et al. (33) also found that angiotensinogen mRNA was colocalized with the astrocyte marker glial fibrillary acidic protein in pineal glands from rats. Angiotensinogen mRNA and angiotensinogen immunoreactivity were both present in glial cell populations of all rat cerebellar layers, especially in the Purkinje and granular cell layers and within the cerebellar nuclei (419). Astroglia as a main source for angiotensinogen synthesis were also shown by other studies (65, 303). In contrast to these findings, some studies have succeeded in demonstrating angiotensinogen also in pure neuronal cultures (269). High levels of angiotensinogen and its mRNA are found in hypothalamus and brain stem (268, 427, 572). In dog brain, ~95% of recovered angiotensinogen has been reported to be extracellular (466), and Thomas et al. (709) have demonstrated the distribution of angiotensinogen in the rat brain by immunocytochemistry. This is supported by findings that the cerebrospinal fluid of dog and rat contains considerable amounts of angiotensinogen (455, 609).

The impermeability of the blood-brain barrier and blood-cerebrospinal fluid barrier for ANG II was reported by several authors (329, 610, 747). With the use of radioimmunoassay and HPLC analysis, ANG II was found with the highest levels in the hypothalamus, pituitary, and cortex but also in the superior colliculus, brain stem, amygdala, cerebellum, hippocampus, and olfactory bulb of rats (547). In this context, a gender difference between the level of ANG II in male and female rat brains was described. ANG II levels in male rat brains are consistently higher in all tissues except the olfactory bulb. ANG II immunoreactivity was found in neurosecretory magnocellular cell groups of the hypothalamus, particularly in paraventricular nucleus, supraoptic nucleus, as well as different accessory cell groups using immunocytochemical methods (574). This suggests the existence of an intrinsic hypothalamic and a hypothalamo-neurohypophysial system, since the fibers from the neurons of the accessory nuclei project directly to adjacent blood vessels and do not comigrate with the hypothalamo-neurohypophysial fiber pathway (574).

Schiavone et al. (612) reported that the peptide ANG-(1−7) is the major metabolite of dog brain stem homogenates both in the absence and in the presence of angiotensin-converting enzyme inhibitors. These data raised the possibility of a functional role for ANG-(1−7) in the brain. Subsequent studies in the in vitro hypothalamo-neurohypophysial system of the rat demonstrated that ANG-(1−7) is equipotent with ANG II in its activation of arginine vasopressin (AVP) release (193, 203). ANG-(1−7) was found in rat neurons in the supraoptic nucleus, and in the anterior, medial, and lateral parvocellular, and posterior magnocellular subdivisions of the paraventricular nucleus (363). Three-dimensional reconstructions showed that cells immunoreactive to ANG-(1−7) and vasopressin were specifically codistributed in the supraoptic nucleus and in the posterior magnocellular subdivision of the paraventricular nucleus, suggesting that ANG-(1−7) and vasopressin are colocalized and coreleased.

E) ANGIOTENSIN RECEPTORS. The presence of receptors for ANG has been amply demonstrated in rat brain tissue (662, 717−720).

Strong AT1B receptor mRNA expression coincided with low AT1A receptor mRNA expression in the anterior pituitary of rat, and both receptor mRNAs were detected in the hippocampus, cingulate cortex, and choroid plexus of rat brain (325). Baltatu et al. (33) have detected that in rat pineal gland AT1B mRNA expression exceeded the expression AT1A mRNA and was colocalized with the pinealocyte-specific tryptophan hydroxylase. AT1A receptor mRNA but not AT1B or AT2 mRNA as expressed in the subfornical organ and paraventricular nucleus of the rat hypothalamus, and AT1B as well as AT1A mRNA were found in the cerebral cortex and hippocampus of rat; conversely, AT2 mRNA, but not AT1A or AT1B, was expressed in the medial geniculate nucleus and inferior olive of rat (324).

AT2 mRNA was found in several thalamic nuclei, medial geniculate nuclei, the nucleus of the optic tract,
the subthalamic nucleus, the interposed nucleus of the cerebellum, and the inferior olive of rat (323).

Expression of AT_{1B} receptor mRNA was present in 33% of rat anterior pituitary cells, predominantly expressed by lactotropes and to a lower degree by corticotropes and is not detectable in somatotropes, mammotropes, gonadotropes, or thyrotropes (385), in contrast to the distribution of angiotensinogen, prorenin, and renin in the anterior pituitary.

High ANG II binding sites were found in medulla, hypothalamus, and circumventricular organs of rat (222). Moderate to strong AT_{1A} labeling was found in the anterior olfactory nucleus, the piriform cortex, and the nucleus of the lateral olfactory tract (384). With the use of immunohistochemistry, the AT_{2} receptor was detected in the locus ceruleus, bed nucleus of the accessory olfactory tract, and cerebellum in association with the Purkinje cell layer, and the deep cerebellar nuclei of the rat brain further structures were hippocampus, limbic structures, thalamic areas, and hypothalamic areas (570). In the mouse brain AT_{1} and AT_{2} receptor distribution was similar to that of the rat, with some notable exceptions, such as the presence of AT_{1} but not AT_{2} receptors in the locus ceruleus and the expression of AT_{1} receptors in the caudate putamen (264). MacGregor et al. (430) have shown that the ANG II binding sites in all forebrain, midbrain, pontine, medullary, and spinal cord sites, as in the small and large arteries in the adjacent meninges and choroid plexus of the human was of the AT_{1} subtype. And both AT_{1} and AT_{2} receptors occur, in contrast to the rat, in the molecular layer of the cerebellum. AT_{1} immunoreactivity was detected in several hypothalamic nuclei, substantia nigra, locus ceruleus, nucleus tractus solitarius, ventrolateral medulla, pontine nuclei, and inferior olivary nucleus of the human brain (44). Brain ANG II receptors are differentially regulated by a number of stimuli. Chemical lesions of the young rat inferior olive reduced ANG II binding to AT_{2} receptors and AT_{1} receptor mRNA levels in this area by 50% and produced a similar decrease in AT_{2} receptor binding in the molecular layer of the cerebellar cortex (322). The extent of binding reduction was similar 3 and 7 days after the lesion. Interestingly, the authors have detected only AT_{2} binding sites in the molecular layer and not AT_{2} receptor mRNA. The lesions did not change ANG II binding to AT_{1} receptors in the molecular layer or AT_{1} receptor mRNA levels in Purkinje cells; also AT_{2} receptor binding and AT_{2} receptor mRNA levels in the deep cerebellar nuclei were not affected according to the authors. A weak, diffuse cytoplasmatic AT_{1} receptor-like immunoreactivity was observed in almost all the catecholaminergic cell bodies of the A2, C1, C2, and C3 cell groups of rat, except those of the A1 cell group (801). In addition, the AT_{1} receptor-like immunoreactivity was seen in noncatecholaminergic neurons. With the use of electrophysiological (287) and autoradiographic studies it was reported that astrocytes of rats express AT_{1} receptors. AT_{2} receptors were detected immunohistochemically within the hypothalamic paraventricular and the supraoptic nuclei of the rat brain, and more specifically, in neurons also containing vasopressin (642). But in those structures there is a lack of AT_{2} binding using receptor autoradiography, suggesting that AT_{2} receptors are transported to the posterior pituitary.

Using a gene targeting approach in mice, Davisson et al. (137) have proposed that the AT_{1A} receptor is mainly involved in central blood pressure control while the AT_{1B} receptor is responsible for mediating the drinking response.

AT_{1} receptors as ligands for ANG IV are located in brain areas implicated in memory acquisition and retrieval, stress, and spatial learning (785). Specific ANG IV, ANG-(3–8), binding sites were found in the forebrain, in particular in the cortex, the hippocampus, the amygdala, the hypothalamus, and the hypothalamic nuclei of mice (749). Rat AT_{2} receptors were found in cerebral cortex, piriform cortex, hippocampus, habenulae, colliculi, septum, periaqueductal gray, several thalamic nuclei, the arcuate nucleus of the hypothalamus, and cerebellum (575). The authors have shown that after intracerebroventricular (ICV) injection of ANG IV, Fos-like immunoreactivity was present in the hippocampus and piriform cortex and that this effect is only blocked by a specific AT_{4} receptor antagonist. In sheep spinal cord, a high density of ANG IV binding sites was localized to the perikaryon and processes of all somatic motor neurons, the autonomic motor neurons in the lateral horns of thoracic and lumbar segments and all dorsal root ganglia and also in numerous motor-associated regions at the supraspinale level, with weaker binding observed in the sensory regions (452). In macaca fascicularis brain, AT_{4} receptor binding sites were found in motor nuclei and motor-associated regions as ventral horn spinal motor neurons, all cranial motor nuclei including the oculomotor, abducens, facial, and hypoglossal nuclei, and the dorsal motor nucleus of the vagus, in the vestibular, reticular and inferior olivary nuclei, the granular layer of the cerebellum, and the Betz cells of the motor cortex with moderate receptor density in all cerebellar nuclei, ventral thalamic nuclei, and the substantia nigra pars compacta; abundant AT_{1} receptors are also found in the areas associated with cholinergic nuclei and their projections, including the nucleus basalis of Meynert, ventral limb of the diagonal band and the hippocampus, somati motor nuclei, and autonomic preganglionic motor nuclei (454).

There is still the possibility that we have not yet discovered all relevant ANG II receptors. In the gerbil brain and pituitary, most of the ANG II receptors are different from AT_{1}, AT_{2}, and AT_{4} subtypes (149).

The Mas protooncogene, a heptahelical receptor in search of a specific ligand, has been proposed as a medi-
ator of ANG II-related effects in the brain (15, 311). While it appears unlikely that Mas is a specific receptor for ANG II, it has been suggested as an enhancer of AT_1-mediated effects in the brain (750). Aside from this functional interaction, Mas could also act as a specific receptor for alternative ANG peptides such as ANG-(1–7), which should be verified. Knock-out studies of Mas showed that it plays an important role as a modulating factor in the electrophysiology of the hippocampus (759).

F) FUNCTION. I) Blood pressure regulation. The brain RAS appears to play a prominent role in central blood pressure regulation. There is increased activity of renin in several nuclei of the brain stem and in neurohypophysis of young hypertensive rats when compared with age-matched normotensive control animals (611). The higher renin mRNA concentration in the brain stem of SHR appears to be at least partially determined by the genotype of the renin gene (648). The binding activity of AGE 2 (angiotensinogen gene activating element) and angiotensinogen mRNA levels were higher in the brain of spontaneously hypertensive rats (SHR) compared with controls (500). In hypertensive rats (SHR) there were also a significantly higher numbers of measurable AT_1 and AT_2 receptor subtypes in the hypothalamus compared with controls (252). This is not a secondary phenomenon, since transgenic approaches leading to a permanent reduction of angiotensinogen in the brain show opposite characteristics. Basal systolic blood pressure was significantly lower in transgenic rats with permanent inhibition of brain angiotensinogen synthesis compared with controls (35, 616). Mice lacking the AT_2 receptor gene have an increase in blood pressure and increased sensitivity to pressin release and sympathetic nerve activity but may affect the central sensitivity of the cardiac sympathetic afferent reflex or the baseline hemodynamics, but the baseline of renal sympathetic nerve activity increased during the infusion of ANG II (428). However, chronic ICV infusion of ANG II enhanced the central sensitivity of the cardiac sympathetic afferent reflex via AT_1 receptor. In addition, chronic ICV infusion of ANG II elicited an increase in arterial pressure. In mice, the blood pressure increase elicited by centrally administered ANG II is most likely due to AT_{1A} receptor activation (137). However, the drinking response requires the presence of AT_{1B} receptors.

Increased AT_1 expression in the brain has been suggested as an early marker for the development of hypertension. AT_1 protein levels were higher in the brain stem of the Zucker obese rat (53), a rat model with a predisposition for hypertension. An ICV injection of ANG II in these rats caused a significantly greater increase in blood pressure compared with control animals. Similar changes are seen in other cardiovascular disorders. Higher binding densities for AT_1 receptor were found in the subfornical organ, paraventricular hypothalamic nuclei, and solitary tract nuclei in rats with chronic heart failure compared with controls (803). Thus, in rats with chronic heart failure, AT_1 expression is increased in brain regions that are closely related to water intake, vasopressin release, and hemodynamic regulation.

In rat hypothalamus there is an inhibitory effect on noradrenergic neurotransmission caused by ANG-(1–7) which has been suggested to be mediated by the AT_2 receptor (229). Furthermore, inhibition of NO synthase (NOS) prevented this effect, suggesting the involvement of NOS-related mechanisms. Rats pretreated with Nω-nitro-l-arginine methyl ester (l-NAME), a NOS inhibitor, showed a greater pressure response to ANG II microinjection into the rostral ventrolateral medulla, than in rats without l-NAME treatment (716). Microinjection of the AT_1 receptor antagonist CV11974 into the depressor region within the nucleus tractus solitarii of rat produced greater decreases in arterial pressure, heart rate, and renal sympathetic nerve activity in l-NAME-treated rats than in control rats (188). In addition, ACE mRNA levels in the brain stem were greater in l-NAME-treated rats (188).

The brain RAS is closely linked to central vasopressin actions. The transgenic rats with reduced (−90%) brain angiotensinogen levels described above exhibit a diabetes insipidus-like syndrome producing an increased amount of urine with decreased osmolarity (616). There was also a reduction in plasma vasopressin by 35% in these animals. Kagiyama et al. (330) suggested that the reduction of angiotensinogen in rats plays important roles in vasopressin release and sympathetic nerve activity but may not contribute to the maintenance of arterial pressure in hypertensive rats. ICV administration of ANG II and ANG
III in rats significantly increased renal plasma flow, glomerular filtration rate, urine flow, absolute and fractional excretions of sodium and potassium, and decreased renal efferent nerve activity; in higher levels, it also increased blood pressure (94). ANG II produced antidiuretic effects in a dose-dependent manner when microinjected into the supraoptic and paraventricular nuclei of rats (593). This effect is reduced by pretreatment with a vasopressin antagonist.

Pharmacological inhibition of the brain RAS has beneficial effects in the brain. Intracerebroventricular infusion of captopril significantly attenuates the pressor response produced by systemically infused ANG I (576). Losartan injection into the anterior hypothalamic preoptic area produced a depressor response in SHR and DOCA-salt hypertensive rats but not in normotensive Wistar-Kyoto rats, whereas ANG II injection into the same area produced a pressor response in sham-operated, Wistar-Kyoto rats, SHR and DOCA-salt hypertensive rats, and the pressor response to ANG II was greater in DOCA-salt hypertensive rats and SHR than that in sham-operated rats and Wistar-Kyoto rats (365). There was also a greater release of ANG peptides in the anterior hypothalamic preoptic area of DOCA-salt hypertensive rats as in the anterior hypothalamic preoptic area of sham-operated rats. The ACE inhibitor imidapril reduced edema formation in the cortex, hippocampus, and striatum of rats suffering from stroke (792), and the progression of neurological deficits with loss of learning ability was suppressed. Treatment of stroke-prone SHR rats with perindopril prevents stroke through the suppression of blood pressure elevation and prevention of tissue damage in the brain (762). Nishmura et al. (502) have shown that pretreatment with the AT1 receptor antagonist candesartan protected hypertensive rats from brain ischemia by normalizing the cerebral blood flow response, most likely through AT1 receptor blockade in cerebral vessels and in brain areas controlling cerebrovascular flow during stroke.

II) Drinking and food intake. Epstein et al. (185) discovered that ANG II elicited drinking when centrally administered into the brain of the rats. ICV administration of renin to rats also induced vigorous drinking behavior (790). These effects are reduced when renin-injected rats were pretreated with captopril. Other studies showed that the ICV infusion of captopril completely blocked the drinking response produced by systemically infused ANG I (576). Conversely, a high sodium intake increased the renin mRNA in the rat hypothalamus, despite the lowering in renal renin mRNA (501) showing the independent regulation of the brain RAS by this stimulus. Chronic ICV infusion of ANG II into the dog elicited an increase in water intake (428). ICV infusion of losartan reduced postprandial drinking in sheep but did not affect food intake, while the intravenous administration had no effect on drinking (440).

In cattle, ICV infusion of losartan induced a dose-dependent inhibition of the high water intake caused by water restriction or by ANG II infusion but did not affect salt appetite (51). Treatment with saralasin, a nonselective ANG II receptor antagonist, into the median preoptic nucleus area of rats produced significantly attenuated saline intake, but had no effect on water intake (703). In addition, the direct injection of ANG I into the rat subfornical organ stimulated drinking, which was blocked by losartan pretreatment (791). Prior administration of losartan into rat supraoptic nucleus decreased water and sodium intake induced by the injection of ANG II into the medial septal area of water-deprived rats (18). This effect was not seen by administration of PD123319, an AT2 receptor antagonist, applied directly into the supraoptic nucleus. AT2 receptor stimulation inhibits drinking response and vasopressin release following centrally administered ANG II in rats (283).

Dehydration differentially upregulates angiotensinogen and AT1A receptor mRNA formation in distinct regions of the rat subfornical organ (36). ICV administration of losartan completely blocked water intake caused by ICV administration of ANG II, and following food deprivation, food intake was reduced by PD123319. Interestingly, neither water intake after water deprivation nor sodium intake after sodium depletion were altered by losartan or PD123319 (768). ANG II microinjected into the organum vasculosum prelaminar terminalis increased water consumption but did not induce intake of a hypertonic NaCl solution. Again this effect was antagonized by losartan (181). The dose-response curve for water intake after ICV injections showed a higher sensitivity to ANG II in transgenic rats with specific downregulation of astroglial synthesis of angiotensinogen compared with controls (458).

Water deprivation upregulates AT1 binding and mRNA in rat subfornical organ and anterior pituitary (598). Following direct stimulation of the rat paraventricular nucleus with hyperosmotic solutions, concentration-dependent increases in ANG II immunoreactivity were found (561). The centrally induced natriuresis and blood pressure response to hypertonic saline that is ICV administered involves AT1 receptors in the subfornical organ of rats (580).

III) Function of the blood-brain barrier. The local RAS in the brain is thought to play a functional role in the maintenance of the blood-brain barrier. Angiotensinogen but not renin levels in the brain appear to be relevant for this function, since a decrease in density in granular layer cells of hippocampus and an impaired blood-brain barrier function which is seen in angiotensinogen-deficient mice, whereas renin-deficient mice do not show this phenotype (799). Other studies in knock-out mice came to similar conclusions. Astrocytes of angiotensinogen knockout mice had very attenuated expression of glial fibrally
higher AT1 receptor binding in most of the regions inside the blood-brain barrier in transgenic rats compared with controls. These data are in contrast to reports by Rose et al. (581) who suggested AT1 receptor-mediated uptake and transport of ANG II at the site of the bovine blood-brain barrier. This has been questioned since there is no evidence that angiotensins cross the blood-brain barrier and penetrate noncircumventricular organ structures (256).

Monti et al. (458) found functional upregulation of the AT1 receptors inside the blood-brain barrier in a transgenic rat line with specific downregulation of astroglial synthesis of angiotensinogen. The authors have found a higher AT1 receptor binding in most of the regions inside the blood-brain barrier in transgenic rats compared with controls. In contrast, in the circumventricular organs investigated, AT1 receptor binding was significantly lower in transgenic rats.

IV) Central actions on the reproductive system. In the pituitary of female rats, mRNA levels for angiotensinogen were increased by 45% following estrogen administration (346). These authors also demonstrated that estradiol caused a 30–40% reduction in the levels of AT1 mRNA in pituitary and hypothalamic-thalamic areas of the female rat. A reduction in ANG II binding to AT1 receptors in the pituitary and the subfornical organ was measured following estrogen treatment. AT1A receptor mRNA expression is induced by estrogen-progesterone in dopaminergic neurons of the female rat arcuate nucleus (326). ANG II immunoreactivity into rat cerebrospinal fluid was greater on proestrus compared with diestrus day 1, and in the overall comparison of ANG II immunoreactivity was released from the preoptic-anterior hypothalamic area, significantly more ANG II immunoreactivity was released on the day of proestrus versus diestrus day 1. ANG II increased prolactin release and produced an immediate spike of intracellular Ca2+ followed by a plateau in cells from rat pituitary (158). These effects were blocked by losartan but not by PD123319, confirming the functional link of the system.

V) Induction of transcription and translation. Central RAS stimulation induces a program of transcription factors commonly associated with the regulation of neuroplasticity. ICV administration of renin to rats induced Fos and Egr-I immunoreactivity in the subfornical organ; median preoptic, supraoptic, and paraventricular nuclei; area postrema, nuclei of the solitary tract; and lateral parabrachial nuclei (790).

Similar findings have been described by Lebrun et al. (378), who after ICV ANG II infusion in rat brain found a dose-dependent expression of c-Fos and Krox-24 in the subfornical organ, the median preoptic area, and the paraventricular nucleus and supraoptic nucleus of the hypothalamus. Furthermore, FosB expression was induced after ICV injection of ANG II in the median preoptic area and paraventricular nucleus, whereas c-Jun expression was restricted to the median preoptic area, subfornical organ, and paraventricular nucleus, and JunB was only induced in the median preoptic area and subfornical organ. Losartan prevented the ANG II-induced immediate early gene protein expression. Also the direct injection of ANG I into the rat subfornical organ induced Fos immunoreactivity in the anterior third ventricle and in the vasopressin neurons of the supraoptic and paraventricular nuclei, which was blocked by losartan pretreatment (791). ANG II produced an upregulation of the AT2 mRNA level in rat cortical neurons (645). This was blocked by an AT2 receptor antagonist. It has also been shown that growth hormone upregulates AT1A receptor in primary cultures of rat astrocytes by a transcriptional mechanism (788). AT2 stimulation promotes differentiation and axonal regeneration and inhibits proliferation of neuronal cells (421, 443, 674). ANG II induces increased elongation of neurites and cell migration in microexplants cultures of the cerebellum from 3-day-old rats via activation of AT2 receptors (112). Furthermore, the authors have shown an increase in expression of neurite-specific β-III-tubulin and microtubule-associated proteins tau and MAP2 and that AT1 receptor inhibits the AT2 effect. In another study the same authors (113) were able to demonstrate that ANG II, via the AT2 receptor, induces nitrite formation from NO which was abolished by L-NAME. Furthermore, the treatment of the neuronal cell culture with SNAP, an exogenous source of NO, induced the same morphological differentiation. Other mechanisms that could be responsible for AT2-related effects include stimulation of the serine/threonine phosphatase A2 and inhibition of MAP kinases Erk1 and Erk2 (292). AT2 receptor upregulation may be involved in apoptosis and tissue repair after stroke, since AT2 receptor gene expression was found to increase in the infarct cortex of rats with permanent occlusion of the middle cerebral artery (810). Additional data emerged that lend further support to the idea of the involvement of local RAS in tissue death after stroke. A recent study reported a decrease in infarct volume and cerebral edema of rats after reperfusion injury when animals, without affecting systemic hemodynamics, were treated with an ACE inhibitor (289).

Neurons cultured from newborn rat hypothalamus and brain stem undergo apoptosis when exposed to ultraviolet (UV) radiation. This effect is enhanced by ANG II and can be blocked by the AT2 receptor antagonist PD123319 (644). Additionally, ANG II enhanced the UV radiation-induced decrease in the levels of the DNA repair enzyme poly-(ADP-ribose) polymerase. The MAP kinases in the rat brain neuronal cultures are activated by AT1 receptors and inhibited by AT2 receptors, again pointing to dual functions of these receptors (202).

VI) Temperature regulation. AT2-deficient mice exhibited a lower body temperature compared with controls.
and show smaller hyperthermia after intraperitoneal injection of interleukin-β and greater increase in body temperature and physical activity after nonimmunological stress (766), suggesting a role for the brain RAS in temperature regulation.

VII) Motor control. Either bilateral or unilateral microinjections of ANG II into the hippocampal CA1 area of rats affected locomotor activity in a dose-related U-shaped manner (41, 42). The effect was more pronounced when ANG II was microinjected into the left CA1 area.

VIII) Visual system. Microinjection of ANG II in the superficial layers of the superior colliculus of rat yielded a strong reduction in the amplitude of visual evoked potentials in a dose-related manner (114). Merabet et al. (446) have shown that in rat this is predominantly mediated by AT1 receptor (446).

IX) Behavior and emotions. Belcheva et al. (43) suggested that ANG II facilitates learning and memory of rats, especially when microinjected into the left CA1 hippocampal area. Winnicka (777) has detected that bilateral 6-OHDA lesions to rat central amygdala (CA) totally abolished the facilitatory effect of ICV infusion of ANG II on the retrieval process in a passive avoidance situation (777). ANG II significantly increased the amplitude of field potentials, of the rat lateral nucleus of the basolateral amygdala, induced by the electrical stimulation, whereas ANG IV caused a significant decrease in the amplitude of field potentials (748). Wright et al. (786) suggested that the brain ANG IV-AT4 system plays a role in the formation of spatial search strategies and memories, since the AT4 receptor is heavily distributed in the CA1-CA3 regions of the hippocampus, and chronic ICV administration of AT4 agonist (Norleucine1-ANG IV) facilitated the rate of acquisition to solve the spatial learning task. ANG II and ANG(3–7) improved object recognition in sham-operated rats to nucleus accumbens septi and to nucleus septi lateralis, and bilateral 6-OHDA lesions to nucleus accumbens septi totally abolished and to nucleus septi lateralis attenuated the facilitatory effect of both ANG peptides on object recognition (778). This suggests that dopaminergic projection to the septum mediale mediates facilitatory effect of angiotensins on recognition memory in rats (778).

Mice lacking angiotensinogen displayed the reduction of “depressive-like” behavior in the behavioral despair swim test and spontaneous locomotor activity diminished; however, they showed no anxiogenic-like or memory-deficit behavior, and there was no change in the pain threshold (515). This was confirmed in other studies where mice lacking angiotensinogen show no differences compared with controls in anxiety and learning (760). However, transgenic rats with diminished angiotensinogen production showed more signs of anxiety than control animals. An effect most likely due to the simultaneously observed decreased in brain 5-HT levels, suggesting a cross-link to the serotonergic system (746). Sakagawa et al. (591) have shown that the pain threshold was significantly lower in AT2-deficient mice, compared with findings in wild-type mice, and that the fluorescence intensity of β-endorphin in the arcuate nucleus of the medial basal hypothalamus was significantly lower in AT2-deficient mice, compared with findings in wild-type mice. Furthermore, the authors found that the AT2 receptor does not influence learning behavior and brain edema formation.

Dexamethasone injection in rats caused an increase in AT1 receptor mRNA in the hypothalamic paraventricular nucleus, and AT1 mRNA is also increased after exposure to stress paradigms associated with activation of the hypothalamic-pituitary adrenal axis (6).

2. Peripheral nervous system

A) SYMPATHETIC NERVOUS SYSTEM. ANG II binding sites were localized and quantified in rat stellate and superior cervical ganglia by quantitative autoradiography (85). High densities of AT1 receptors in sheep (516) and ANG II immunoreactive terminals in guinea pig and rat (215, 414) occur in the intermediolateral cell column of the spinal cord and the central autonomic area.

A direct relationship between the effect of ANG II and the sympathetic nervous system is likely since sympathectomy or reserpine treatment attenuates the blood pressure response to ANG II. Intrathecal administration of ANG II increases sympathetic vasomotor nerve discharge and blood pressure in rats (688). The interaction of ANG II with postganglionic nerve terminals was shown by Day and Owen (138). In addition, Lewis and Reit (406) have shown that ANG II potentiates sympathetic transmission through the sympathetic ganglia of the cat.

ANG II is able to increase the excitability of superior cervical ganglia cells (640) and cells from sympathetic region of the thoracic and lumbar spinal cord or rat (405). Cox et al. (117) have shown that ANG II and ANG III were able to facilitate the action potential-induced release of norepinephrine via a prejunctional AT1 receptor in mouse atria and spleen. In the rat, ANG II caused an increased sodium and water absorption that is mediated by increased norepinephrine release from sympathetic neurons (400).

Uregulation of ANG II release and angiotensinogen mRNA expression can be induced by high-frequency preganglionic stimulation at the canine cardiac sympathetic ganglia (369). The resulting positive chronotropic responses were inhibited by the nonpeptide AT1 receptor antagonist forasartan or the ACE inhibitor captopril, while the increase in heart rate elicited by postganglionic stellate stimulation could not be inhibited by these compounds (369).
B) PARASYMPATHETIC NERVOUS SYSTEM. In vitro autoradiographic techniques demonstrate a high density of AT₁ receptors in intracardiac ganglia and conduction systems of rat heart (14, 590), but it is not known if they are localized presynaptically or postsynaptically. Allen and co-workers (11, 12) detected that ANG II receptors are transported peripherally in the rat vagus nerve and may well be localized on the terminals of parasympathetic preganglionic neurons whose cell bodies lie in either the nucleus ambiguus or dorsal motor nucleus of vagus in the medulla. Diz and Ferrario (166) provided data that reveal the existence of a mechanism for the bidirectional axonal transport of ANG II binding sites in the cervical portion of the vagus nerve of dogs.

Potter (556) has shown that ANG II inhibits release of acetylcholine from the preganglionic neurons via a presynaptic mechanism in intact dogs and in isolated guinea pig atria.

Ikegami et al. (300) showed that ~70% of hamster submandibular ganglion neurons responded to ANG II with persistent depolarization. This effect is mediated by AT₁ receptor subtypes. The AT₂ receptor has been implied in neurotropic effects in the peripheral nervous system, since it could be demonstrated that AT₂ effects promote axonal regeneration after optic nerve crush in a tissue culture model (421).

C) SENSORY NEURONS. There are suggestions that AT₁ receptors are associated with a range of afferent nerves. AT₁ receptors were detected on cell bodies in nodose ganglion, dorsal root ganglia, and the primary central terminal regions of these neurons, the nucleus of the solitary tract and dorsal laminae of the spinal cord of several species (10, 11, 516). It has also been suggested that AT₁ receptors occur in the caudal nucleus of the spinal trigeminal tract in the medulla (13) and that they may be associated with the terminals of trigeminal sensory neurons.

D. Reproductive Tract

1. Female reproductive tract

As reviewed by Hagemann et al. (249), renin has been detected in the uteroplacental unit of many species. Skinner et al. (660) showed that all parts of the human uteroplacental unit contain renin, with the highest concentrations found in the fetal membranes (chorion and amnion) and decidua. In humans, prorenin is the major form of renin in the uteroplacental unit (332, 765). Secretion of prorenin from the human uteroplacental unit (57, 388) and the corpus luteum (151) may explain the increased plasma prorenin concentrations in pregnant women, which is more pronounced in the first trimester (632).

A) OVARY. A schematic representation of the localization of RAS components in the ovary is shown in Figure 3.

FIG. 3. RAS in the ovary. Renin, synthesized and secreted by theca cells, leads to local ANG production. Two possible ways in which ANG II may modulate atresia in ovaries has been suggested: 1) ANG II is acting on the AT₂ receptor within the same follicle by an autocrine mechanism leading to apoptosis and atresia; and 2) ANG II is secreted by preovulatory follicles causing apoptosis in adjacent atretic follicles (paracrine mechanism). Follicular fluid contains high levels of renin and ANG II.

1) Renin. Ovarian renin mRNA was detected by several authors (304, 341, 411). Renin mRNA is expressed in the corpora lutea of the rat but not in theca or granulosa of follicles. Follicle-stimulating hormone stimulates renin mRNA expression in the ovary of rat (342) and monkey (304).

The ovary is the major source of plasma prorenin in women (230). The rat ovary contains a moderate amount of prorenin and renin activity, which are increased by pregnant mare serum gonadotrophin (497). Renin is differentially expressed across species borders. Bovine corpora lutea express high levels of renin activity, while porcine corpora lutea have very low levels of renin activity (496). In the cow, ovarian follicles contain prorenin and renin, with the highest level of prorenin expression in atretic follicles (469, 624). Lightman et al. (411) detected renin mRNA in the gonadotropin-stimulated rat corpus luteum using in situ hybridization histochemistry. No positive signal was detected in theca or granulosa cells. Howard et al. (290) suggested that renin activity, which varies with the estrous cycle, arises from theca or interstitial cells of the rat ovary. As mentioned above, Itskovitz et al. (304) could detect renin mRNA in the theca of...
human chorionic gonadotropin (hCG) stimulated monkey ovaries but not renin-like immunoreactivity, suggesting that the product is immediately secreted. Unlike this finding in the human, renin-like immunoreactivity has been observed in theca, stroma, luteal, and granulosa cells (518, 519). Renin-like activity has also been found in human follicular fluid (412).

II) ACE. ACE is abundant in the rat ovary (126). Rat ovarian ACE is membrane bound and is present in the germinal epithelium, capsule of the corpora lutea and in many, but not all, follicles (669). In the human ovary, ACE activity increases with age (187), which could be a result of the age-dependent reduction in the number of ovulating follicles rather than a direct regulatory effect.

III) Angiotensinogen, ANG I, and ANG II. Angiotensinogen mRNA has been shown to be expressed in the ovary of rat and mouse (511, 701). By immunocytochemistry, Thomas and Sernia (710) have found angiotensinogen activity in specific populations of granulosa cells of the rat ovary and, to a lesser extent, in germinal epithelium, stroma, and luteal cells. The angiotensinogen presence appears to be greater in preovulatory follicles than in atretic follicles. ANG II has been found in human follicular fluid (124, 412). Phillips et al. (546) reported that the ovary contained the fourth highest level in 13 tissues examined.

IV) Angiotensin receptors. The presence of AT1A mRNA but not AT1B mRNA could be demonstrated in the mouse ovary by Burson et al. (68). For the most part, follicles that contain ACE also contain ANG II receptors. In the rat, the follicular ANG II receptors are of the AT2 subtype and are located primarily on the granulosa and theca interna cell layers of atretic follicles (135, 560, 667); bovine ovaries contain also predominantly AT2 receptors (64, 496). Rabbit ovaries express both AT1 and AT2 receptors, with the AT1 receptor being localized to the theca and stroma and the AT2 receptor being expressed on the granulosa cells (804). In contrast, Feral et al. (194) reported that only the AT1 receptor subtype was present in the rabbit ovary and that it was localized on preovulatory follicles. Autoradiographic receptor studies indicate that the AT2 receptor subtype is the predominant ANG II receptor subtype in the mouse ovary, with only a low level of AT1 receptor expression (668).

B) UTERUS. I) Renin. Renin mRNA has been reported in the endometrium, choriodecidua, and the fetal part of the placenta (249) but not in the myometrium (299). Via immunocytochemistry, Raju and Lee (566) demonstrated renin in the human endometrial glandular epithelium. During late pregnancy, the uterus is also a source of plasma prorenin (383).

II) ACE. The rat uterus expresses a low but detectable level of ACE (126), and Moeller et al. (451) found ACE activity in high concentrations in the blood vessels of the pregnant and nonpregnant sheep reproductive system and on the epithelial cells of the fallopian tubes of the nonpregnant sheep. ACE immunoreactivity showed a cyclic variation in the human endometrium with the highest expression in the late secretory phase at the menses (410).

Furthermore, the uterus contains cathepsin D (599) and chymase (731), giving rise to possibilities for alternative pathways for ANG II formation in the uterus.

III) Angiotensinogen, ANG I, and ANG II. Angiotensinogen mRNA is expressed in spiral artery smooth muscle in human uterine deciduas (459), but the stroma of the uterus itself does not appear to produce angiotensinogen (218, 299). Immunreactive ANG II was found by Naruse et al. (479) in human uterus. Rat uterus also contains immunoreactive ANG II (154). ANG II-like immunoreactivity was detected in the glandular epithelium and stroma, whereas in the secretory phase intense immunoreactivity was seen in the perivascular stromal cells around the endometrial spiral arterioles.

IV) Angiotensin receptors. Radioligand binding assays were used to demonstrate for the first time radioligand binding to an ANG receptor in uterine tissue (413). In addition, the first study to demonstrate ANG II receptor subtypes showed that the human uterus contains primarily the AT2 receptor subtype (771). ANG II receptors are also present on uterine glands and at a low level in the endometrium of the rat (668). Similar data were provided for the mouse where ANG II receptors are abundant in the mouse uterus and primarily of the AT2 subtype. Interestingly, in homogenates of the mouse uterus, the AT1 receptor predominates, which could be of vascular origin. Since the connective tissue has been removed in this study the authors suggested that AT2 receptor binding seen in receptor autoradiographic studies is attributable to binding to connective tissue rather than on other components of the uterus. Autoradiographic studies in the mouse suggest that cervical AT2 receptor density is very high. Again, as seen in the uterus, homogenates of mouse cervix have relatively low levels of ANG II receptor binding.

In the sheep uterus, AT1 and AT2 receptors are present in the endometrium and myometrium, respectively (451). In late term pregnant sheep, myometrial AT2 receptors are replaced by AT1 receptors. In humans, ~90% of the ANG II receptors in the myometrium of nonpregnant women are of the AT2 subtype (115). The density of the ANG II receptors in the uterus of near-term pregnant women decreases by >90%, reflecting more than a 95% loss of AT2 receptors.

C) OVIDUCT. I) ACE. ACE activity is present in the oviduct of chicken and pig and is localized in epithelial cells of the oviduct in sheep (198, 451, 456).
II) Angiotensin receptors. Receptors for ANG II are present in the human fallopian tube (600). While both AT$_1$ and AT$_2$ subtypes are present, the functional response to ANG II, enhancement of tubal ciliary-beat frequency, was mediated by the AT$_2$ subtype. Receptor autoradiographic studies of ANG II receptor binding in the mouse oviduct indicate that they contain AT$_2$ receptors, but at concentrations that are considerably lower than in the uterus (668).

D) Function. I) Ovary. The RAS has been proposed as a mediator of ovarian function and follicle maturation. Mukhopadhyay et al. (468) have shown that prorenin secretion from cultured bovine theca cells can be stimulated by luteinizing hormone. Hagemann (247) suggested that secretion of prorenin from the ovary into the bloodstream is unique to humans. This group could also demonstrate that luteinizing hormone stimulates renin activity in the follicular fluid of heifers (248). Also gonadotrophin-induced stimulation of renin synthesis and release from theca cells of bovine (63) and monkey follicles (304) has been reported. Gonadotropins are able to stimulate renin formation in the corpora lutea of the rat (155). The authors suggested that angiogenesis of developing corpora lutea was due to the activation of the RAS in ovarioly follicles. However, the report that ANG II receptors are not present on most preovulatory follicles (135) would argue against a significant direct role of follicular fluid ANG II in this angiogenic process. Moreover, recent observations suggest that vascular endothelial growth factor is a primary angiogenic factor in the rat corpora lutea (199, 668).

Fernandez et al. (196) observed that estrogen concentration and renin activity in human follicular fluid were positively correlated, and others (305) have reported that a high level of prorenin was associated with high levels of testosterone in human immature follicle. ANG II is able to induce estrogen release by in vitro pregnant mare serum gonadotrophin stimulated immature rat ovaries, suggesting that ANG II promotes follicular maturation (559). In contrast, Morris et al. (467) suggested that ANG II inhibited estradiol (tonic inhibition by endogenous ANG II) and increased progesterone formation in human cultured luteinized granulosa cells and also increased testosterone in cultured rabbit theca cells in response to hCG (195).

Ovulation induced by pregnant mare serum gonadotropin in either immature rats or a perfused rat ovary system was inhibited in the presence of an ANG II receptor antagonist. This inhibition could be overcome by administration of an excess of exogenous ANG II (539, 544). In gonadotropin-treated rabbits, the nonselective ANG II receptor antagonist saralasin is able to inhibit ovulation (589). This effect could not be shown by inhibition of ACE (543). Kuji et al. (366) reported that ANG II facilitates follicular development and ovulation in the rabbit via the AT$_2$ receptor, since an AT$_2$ receptor antagonist (PD123319) inhibited hCG- and ANG II-induced ovulation and oocyte maturation in vitro, as well as ANG II-induced estrogen and prostaglandin formation. But others (476, 539) using the same ANG II receptor antagonist, or an ACE inhibitor were unable to fully replicate the inhibition of ovulation. Steele et al. (677) also saw no impairment of ovulation in rats that were systemically administered an ANG II antagonist or an ACE inhibitor. The treatment of women of child-bearing age for hypertension with the ACE inhibitor enalapril did not affect normal periodicity of plasma gonadotrophins, gonadal steroids, or plasma prorenin (715). Life-long application of ACE inhibitors to rats did not impair fertility (787). In contrast to this local situation, there was a profoundly reduced ovulation by IVC administration of ANG II receptor antagonist or ACE inhibitor (677).

II) Uterus. Hagemann et al. (249) suggested that the local function of ANG II on the uterus may be to control uterine blood flow during pregnancy.

As might be expected, the contractile actions of ANG II in the human uterus are mediated by AT$_1$ receptors (115, 619). The contractile response of the rat uterus to ANG II and both AT$_1$ and AT$_2$ receptor density vary considerably over the estrous cycle (668).

2. Male reproductive tract

In the male reproductive system, local RAS function is thought to be relevant for fertility. This system is regulated independently from the plasmatic RAS, and the blood testicular barrier prevents that ACE inhibitors and AT$_1$ blockers affect fertility.

A) Testes. A representation of the testicular RAS is shown in Figure 4.

I) Renin. Renin mRNA was detected in mouse testis by hybridization using cDNA and cRNA (173, 179, 522, 531) and RNase protection analyses (448). Further studies (153) have shown the Leydig cells as an origin of this tissue renin mRNA. Renin, ACE, ANG I, ANG II, and ANG III were detected by both HPLC and radioimmunoassay in purified Leydig cells from rat (523). Naruse et al. (478) did provide immunohistological evidence for renin in human Leydig cells.

II) ACE. The ACE gene encodes for two ACE proteins, the somatic ACE enzyme that has two identical lobes and the testicular ACE molecule consisting of a single lobe. The testicular ACE mRNA is transcribed from the ACE gene by the tissue-specific choice of an alternative transcription initiation site as well as an alternative polyadenylation site (291, 293, 367, 707). Bernstein et al. (48) have demonstrated that mouse testicular ACE (tACE) is encoded by a smaller mRNA (2,500 bases) compared with the somatic ACE mRNA species found in other organs such as kidney or lung (4,900 and 4,150...
bases). Molecular cloning of the human tACE cDNA indicates that the mRNA codes for 732 residues (vs. 1,306 in endothelium) (375). tACE is equally active despite the fact that it is translated from a shorter mRNA. Oligonucleotide probes derived from tACE cDNA demonstrated expression of mRNA only in the testes and not in other organs (772). Thus the tACE is highly tissue specific. Leydig cells have been initially suggested as the primary source of ACE in the testis (523), but further investigations have confirmed that germinal cells are the origin of the tACE activity. tACE is expressed at high levels by male germ cells during spermiogenesis while somatic ACE is also expressed in other testicular cells. The regulation of tACE is tissue specific. Systemically administered ANG II decreased ACE mRNA levels in the lung and testis, but unlike the pulmonary ACE activity, the tACE activity shows negligible changes, suggesting that it is caused by different half-lives of mRNA and/or protein (628).

The concentration of ACE in the testis is among the highest in all organs (126). And before washout of seminal fluid, ACE activity is higher in the testis than in epididymis and vas deferens, suggesting secretion of ACE into seminal fluid (282). tACE activity was measured by Hohlbrugger et al. (282), and Berg et al. (45) have shown the existence of pulmonary ACE in rabbit endothelial cells of blood vessels in the interstitial tissue of testis by immunohistochemistry. In contrast to this finding, Strittmatter and Snyder (684) could not detect any existence of ACE in the interstitial tissue of rat testis.

III) Angiotensinogen, ANG I, and ANG II. Angiotensinogen mRNA was detected in testicular tissue in some species. Dzau et al. (174) showed angiotensinogen mRNA in mouse testis, whereas in rat testis angiotensinogen mRNA was undetectable. This goes along with a report by Campbell and Habener (74) who could not detect angiotensinogen mRNA in rat testis using Northern blotting. Only when using a hybridization technique with increased sensitivity (268) low amounts of angiotensinogen mRNA could be detected in rat testis.

IV) Angiotensin receptors. Targeted deletion of the AT1B receptor gene in the mouse has shown high AT1B gene transcription activities in testis (95), including mature and immature spermatic cells. Kitami et al. (347) measured expression levels of AT1A and AT1B receptors in rat testis by PCR and suggested that AT1A mRNA was predominantly expressed.

Aguilera et al. (7) demonstrated the existence of ANG II receptors in mature rat and rhesus monkey testis, indicating that ANG II receptors are located on the Leydig cells. These results were confirmed by demonstrating the localization of the AT1 receptor in rat Leydig cells by immunofluorescence (743).

B) EPIDIDYMIS. The concept of ANG pathways and function in epididymis and sperm is shown in Figure 5.

I) Renin. The search for renin mRNA in this organ was negative. Leung et al. (397) have not found any level in rat epididymis using Northern blot and even more sensitive RT-PCR assays.

II) ACE. ACE activity was measured in rat epididymis (282). Interestingly, epididymal tissue contains both ACE isoenzymes. The somatic ACE isoform was localized in rabbit epididymal blood vessels (45). It could also be shown by these authors that epididymal tubular cells contained pulmonary ACE, suggesting that spermatozoa specifically express tACE. This has been supported by other findings: when spermatozoa were prevented from entering the epididymis by efferent duct ligation, ACE activity in the epididymis was greatly reduced (783).

III) Angiotensinogen, ANG I, and ANG II. Angiotensinogen mRNA was detected via RT-PCR and in situ hybridization histochemistry in rat epididymal epithelium, with the highest levels found in the epididymal tubules of the cauda region (397). Using immunocytochemistry, these authors localized precursor angiotensinogen protein in caput, corpus, and cauda of rat epididymis. The concentrations of ANG I and ANG II are high in the rat epididymal plasma compared with the concentrations in the rete testis fluid and blood serum (783). Also, the level of ANG II in the luminal fluid of the cauda epididymis was...
markedly reduced after efferent duct ligation (783, 808). With immunohistochemical staining, Zhao et al. (808) detected ANG II in rat epididymis with the highest level in the basal cells of the cauda. The same authors suggested a potential of the epithelial cells to secrete ANG II, since ANG II could be detected by radioimmunoassay in the supernatant of cultured endothelial cells.

IV) Angiotensin receptors. Leung et al. (392) provided evidence for the existence of ANG receptors in rat epididymis. Both AT₁ and AT₂ receptors were detected; however, the immunostaining intensity for AT₁ receptors was found to be stronger than that for AT₂ receptors, and the immunostaining for both receptors observed in the fully mature rat epididymis was much more intense than that observed in younger stages. The immunostaining was predominantly localized in the basal region. Grove and Speth (242) have shown that rat epididymis contains functional ANG II receptors. Also, Vinson et al. (743) have found AT₁ receptors in epithelial tissue of rat epididymis. Autoradiographic analysis showed the presence of ANG II receptors also in the rhesus monkey epididymis (7). Light microscopic examination indicated that the binding of radiolabeled ANG II in the epididymis was associated with smooth muscle cells.

C) SEMINAL PLASMA AND SPERMATOZOA. I) ACE. High ACE activity has been determined in human seminal plasma (739). The soluble ACE in seminal fluid resembles the detergent-solubilized lung enzyme more than the detergent-solubilized testicular enzyme (150, 180). The active enzyme in seminal fluid corresponds to the pulmonary polypeptide (45, 372). These authors suggested that this seminal fluid ACE may originate from cells of the epididymal tubules, particularly those of the vas deferens. By autoradiographic visualization with [³H]captopril, Strittmatter et al. (684) have shown high densities of [³H]captopril over spermatid heads, and in the lumen of seminiferous tubules also, the head of the epididymis demonstrates intense grain density at the luminal surface of the epithelium with little luminal labeling by rats. The testicular ACE is expressed at high levels by developing germ cells and is present in mature sperm (774). With the use of indirect immunofluorescence and immunoperoxidase methods, tACE was found in the seminiferous tubules of the testes in spermatocytes containing mature spermatids, and in spermatids within the epididymal tubular lumen in sexually mature, but not in immature, rabbits (45), suggesting that the presence of tACE is dependent on sexual maturation.

II) Angiotensin receptors. AT₁ receptors were found in primary spermatogonia and spermatid tails of rat testis by Vinson et al. (743). These authors were able to confirm this finding in ejaculated rat and human free swimming sperm. Interestingly, they did not find any immunofluorescent activity from the spermatozoa in the lumen of rat epididymis and from sperm isolated from the epididymis. By targeting deletion of AT₁b receptor gene in the mouse, Chen et al. (95) have shown high AT₁ transcriptional activities in mature and immature spermatic cells, suggesting that AT₁a can take over specific functions in these knock-out animals or that other ANG receptors are involved in this process.

D) GLANDULA VESICALIS. I) ACE. High ACE activity has been determined in human vesicula seminalis (739).

E) PROSTATE. I) Renin. Renin mRNA is present in mouse prostate, and the human prostate shows a positive signal after renin immunohistochemistry (478). II) ACE. High ACE activity has been determined in human benign prostatic hyperplasia, whereas normal prostates apparently have low enzymatic activity (282, 739, 802). Other authors (735) have found higher levels in the lateral and posterior prostate compared with ventral prostate in rat, but the activity was still much lower than that in epididymis.

III) Angiotensin receptors. Dinh et al. (164) reported that in human prostate ANG II receptors were of the AT₁ subtype and localized predominantly to periurethral stro-
mal smooth muscle, and the density of ANG II binding sites was markedly reduced in benign prostatic hyperplasia. It has been suggested that this may be due to receptor hyperstimulation by increased local levels of ANG II in benign prostatic hyperplasia.

**F) VAS DEFERENS. I) ACE.** Immunohistochemistry showed strong staining for somatic ACE in cells of the vas deferens in both young and adult rabbit (45), whereas other authors could only describe low ACE activity (735).

**G) SEMINAL VESICLES. I) ACE.** Only low ACE activity was found in seminal vesicles (735).

**H) FUNCTION. I) Testis.** After hypophysectomy renin levels in the rat testis decreased significantly, whereas plasma renin was slightly increased (480), suggesting an alternate regulation of the circulatory and testicular RAS. The immunohistochemical renin staining of rat Leydig cells was suppressed or abolished by hypophysectomy and estrogen treatment and was reduced by gonadotrophin stimulation (520, 525). The treatment of cultured mouse Leydig tumor cell line with human chorionic gonadotropin or bovine luteinizing hormone results in a high increase of renin activity (521). It has to be noted in this context that almost all renin activity is confined inside the cell with only 1–2% of its total activity being present in the culture medium. Okuyama et al. (514) have shown that treatment with hCG increases the plasma renin activity in internal spermatic vein without an increasing of the systemic plasma renin activity. The authors could not observe any release of renin from Leydig cells into testicular blood flow under basal conditions.

There is a positive correlation between testosterone and plasma renin activity in the internal spermatic vein of humans after hCG treatment (525). Treatment with hCG or bovine luteinizing hormone increased also the ANG production in a cultured mouse Leydig tumor cell line (521). It is noteworthy that the major portion of ANG II is released in the culture medium and the majority of ANG I was present in the cell. Together with the mentioned intracellular presence of renin and ACE (523), this supports the possibility of intracellular formation of ANG II.

Hirai et al. (275) could verify that the AT1 and AT2 mRNA gene expression in rat testis is pituitary dependent. After hypophysectomy, the receptor gene expression was significantly increased. Human chorionic gonadotrophin and human menopausal gonadotrophin reduced the AT1 and AT2 mRNA gene expression. Furthermore, the AT2 mRNA expression in rat testis is dependent on developmental stages, since Northern blot analysis showed that mRNA expression of both AT1 and AT2 types decreased with age (334). Aguilera et al. (7) reported also a decrease in ANG II binding during development and that in immature rat a high proportion of ANG II receptors in the testes are located in nondifferentiated mesenchymal cells of the interstitium. Hypophysectomy led to a marked increase in AT1 and AT2 receptor concentration of rat testis with predominant occurrence of AT1 receptors measured by in vitro autoradiography (275). The concentration of rat AT1 and AT2 receptors at protein level is dependent on the developmental stage. Total ANG II receptor binding (predominantly AT2 receptor) in the testis was highest at 1 day of age and decreased gradually thereafter, and the 4-wk-old rat testis contained almost exclusively AT1 receptors (334).

**II) Epididymis.** Anion and fluid secretion is stimulated by a number of peptide hormones, including ANG (782). Previous electrophysiological studies demonstrated a higher potency of ANG II on the basolateral surface in eliciting transient increases in short-circuit current when added to the basolateral as well as to the apical surface of rat epididymal epithelium. ANG II secreted from the basal cells may exerts its effect on electrolyte transport by acting on ANG II receptors on the basolateral membrane of the principal cells, which are largely responsible for the electrolyte transport. Electrophysiological studies using the short-circuit current technique demonstrated a stimulatory effect of basolaterally applied ANG II on the epididymal electrogenic ion transport, suggesting AT1 as the target receptor (394).

**III) Seminal plasma and spermatozoa.** ACE enzyme activity is low in immature animals and increases with the onset of sexual maturity (126, 314). The ACE activity in semen is enhanced by sexual stimulation (282, 313) but is normal in oligospermic men (281). A role of the tACE in capacitation has been suggested by several authors (211, 658). Previous reports have suggested that ACE is released during sperm capacitation (356), which was confirmed after exposure to ACE inhibition that affected the acrosome reaction and the ability of human sperm to penetrate an egg (212).

Knockout technology was used to investigate the role of ACE in male fertility (361). Hagaman et al. (246) have shown with this method that although male mice lacking ACE were infertile, the number of sperm, the viability, motility, capacitation, and induction of the acrosome reaction were normal. Despite these normal functional parameters, very few sperm are found in the uterine tube region, and even those sperm that reach the oviductal ampulla are less likely than normal sperm to fertilize eggs because of their reduced capacity for zone binding. The authors suggested that tACE may play a role for the detachment of sperm from the oviduct epithelium and/or other structures in the female reproductive tract at capacitation. Esther et al. (189) used targeted homologous recombination to introduce a modified ACE allele into a mouse line. Homozygous male mice have markedly reduced blood pressure, severe renal disease, and reduced fertility, however, corresponding to no defect in sperm number, morphology, or motility. Corresponding to this, Ramaraj et al. (567) used transgenic techniques to gener-
ate knockout mice with exclusive expression of tACE using ACE knockoum mice as a background. These animals express ACE only in sperm, since a sperm-specific promoter was used to direct expression of tACE. The background ACE knockout mice lack both isoenzymes (somatic ACE and tACE) and exhibit low blood pressure, kidney dysfunction, as well as male infertility. The transgenic “rescue” by the selective tACE overexpression resulted in normalized fertility. The male fertility defect does not appear to be related to the low levels of ANG II expected in the ACE-deficient mouse. Angiotensinogen-deficient mice, which cannot synthesize ANG II, do not have any defects in male fertility (246, 339). Nagata et al. (477) have also investigated a mouse line lacking angiotensinogen and suggested that female-derived ANG I is also not an essential substrate for tACE.

There are suggestions for a function of AT1 receptors as determinants of sperm motility, since ANG II increases both the percentage of motile sperm and their linear velocity and AT1 receptor antagonists inhibit this action of ANG II (743).

E. Skin

1. Renin

Northern blot analysis showed renin mRNA expression in murine subcutaneous tissue but not in murine dermis and epidermis (653).

2. ACE

One study (685) could detect large amounts of ACE in subcutaneous tissue of the rat by quantitative autoradiography, but it is unclear which cell type expresses ACE in this tissue. It could also be expressed by vascular endothelial cells or be taken up from the circulation. No signal for ACE was detected in the dermal and epidermal layers of rat skin. Steckelings and co-workers (675) pointed out that the absence of ACE in this region as well as in the subcutaneous tissue may not present a problem for local ANG formation, since the skin is abundant in mast cells and that mast cell-derived chymase may act as a substitute for ACE (217, 675). Moreover, more recently, it was shown that mast cells are an additional source of renin synthesis (654), which could be of relevance for the RAS activity and regulation at the tissue level, because of the ubiquity of mast cells in tissues.

3. Angiotensinogen, ANG I, and ANG II

There is only one study suggesting local production of ANG I and ANG II in the skin of captopril-treated pigs (130). ANG I and ANG II levels in the presence of constant intracardiac infusion of $^{125}$I-ANG I and $^{125}$I-ANG II were measured in arterial and venous plasma across cutaneous vascular beds, and these values were compared with plasma renin activity. The data provide a higher level of ANG I in venous plasma compared with the level in arterial plasma, independent of plasma renin activity. This is seen as evidence for local ANG I release; however, the concentration difference could also be caused by a higher local renin activity. Unfortunately, there are no data available that could be considered as evidence for the local synthesis of angiotensinogen.

4. Angiotensin receptors

Radioligand binding studies were performed on cultured human primary keratinocytes and showed binding to ANG II. Interestingly, the AT1 and AT2 receptor antagonists losartan and PD123177 are not able to displace the radioligand. Thus the keratinocytes seem to express an ANG II receptor subtype distinct from AT1 and AT2 receptors (673).

5. Function

A) WOUND HEALING AND REPAIR. Increased ANG II levels were measured during wound healing in rat skin (243, 345), but this is not conclusive evidence for local production because ANG II could also be taken up from plasma into the tissue. Previous studies described a proliferative and antiproliferative effect of ANG II in the skin which appear to be mediated by AT1, AT2, and non-AT1, non-AT2 receptors (105, 485, 524, 673, 681).

Quantitative autoradiography demonstrated that the expression of ANG II receptors was significantly enhanced during experimental wound healing. Young rat skin normally contains mainly AT1 receptors, but during experimental wound healing a selective and localized increase in expression of AT2 receptors occurs (745). This study suggested skin fibroblasts as the primary target cells for ANG II. Other investigators (345) have found significant decreases in the number of AT1 receptors and a modest but not statistically significant appearance of AT2 receptors in adult rat skin during wound healing. This is not necessarily a contradiction and could be an indication for the importance of the proportion or balance of these two receptors. Abiko et al. (3) have shown that an immediate and transient reduction in ANG II receptor expression occurred after wounding rat skin, followed by an increase in the number of the receptors that was maintained for 5–7 days, and that the receptors were predominantly of the AT1 receptor subtype. In a cutaneous repair model, Sun et al. (686) have shown that the appearance of myofibroblasts is associated with ANG II generation which regulates fibrogenesis by AT1 receptor binding. In rat skin slices, ANG II binding to AT1 receptors increases inositol phosphate production, while binding to AT2 decreases inositol phosphate production; thus both receptors play opposite roles (243). It has to be noted that...
it was unclear in the two former studies in which cell type the ANG II receptor regulation and signal transduction occurred (243, 686). Further investigation has suggested a mitogenic effect of ANG II of keratinocytes via a non-AT₁, non-AT₂, ANG receptor (673). ANG-(1—7) binding sites were also characterized in human skin fibroblasts (493).

F. Digestive Organs

1. Salivary glands

A) RENIN. The salivary gland of some mouse strains expresses significant amounts of renin. It is interesting to note that renin production in the salivary gland is particularly high in mouse strains that contain two renin genes (Ren-1 and Ren-2), which are the result of a gene duplication that occurred during evolution (2). Both genes are expressed in most mouse organs such as kidney (204), whereas only Ren-2 is expressed in mouse salivary glands, particularly in the submandibular gland (179, 205). Although the functional significance of this gene duplication is unknown, the study of two-gene mice provided an excellent opportunity to investigate the molecular basis of tissue-specific renin gene expression, and the functional role of the Ren-2 gene has been studied extensively in transfected cells (529), transgenic mice (472), and transgenic rats (471). The latter model is of particular importance in this context, since the transgenic rats with Ren-2 expression suffer from fulminant hypertension and severe end-organ damage. The gene duplication is specific for the mouse, and expression of the rat and human renin genes correlates (159) with that of the mouse Ren-1 gene.

B) ANGIOTENSINOGEN. Low angiotensinogen mRNA levels were detected in the CD-1 male mouse and the C57BL/6 mouse (701) submandibular gland, but like renin mRNA, angiotensinogen mRNA could not be detected in rat submandibular gland (174).

C) RECEPTORS. Using a radioligand binding assay, Matsubara et al. (442) found mainly AT₁ receptor subtypes in rat submandibular organ.

D) FUNCTION. The ultimate role of renin and other components of the RAS in the submandibular gland are not known. It has been associated with mediation of the “fight and flight” reaction. Renin activity in the salivary glands increases with stress, pituitary hormone release, and adrenergic stimuli and is associated with aggressive behavior in mice (557, 775). There have also been indications of a role in saliva production, since ACE inhibition increases overall saliva secretion of humans but does not affect its composition (483).

2. Pancreas

A) RENIN. Renin mRNA was detected in the connective tissue surrounding the blood vessels and in reticular fibers within the islets of human pancreas (695). Renin protein was found in the beta cells of the human islets of Langerhans and in endothelial cells of the pancreatic vasculature.

B) ANGIOTENSINOGEN, ANG I, AND ANG II. Angiotensinogen mRNA and angiotensinogen protein were found in canine pancreas (93). In contrast, Campbell and Habener (74) did not find angiotensinogen mRNA in rat pancreas. The presence of ANG II was reported in canine pancreas by Chappell et al. (93). Also in mouse ANG II-like immunoreactivity was localized predominantly in the endothelial cells of pancreatic blood vessels and the epithelial cells of pancreatic ducts from a subgroup of the vessels and ducts, and with lower intensity in acinar cells and in smooth muscle layers overlying the pancreatic ducts and blood vessels. However, no ANG II immunoreactivity was detected in the islet cells (395).

C) ANGIOTENSIN RECEPTORS. The majority of ANG II binding sites in the canine pancreas could be classified as AT₂ subtypes, although AT₂ subtype was also detected (92). The same study has reported that the binding sites were localized over islet cells, acinar and duct cells, as well as the pancreatic vasculature. ANG II binding sites were also found in islet cell membranes and the exocrine pancreas of the rat (224). Another study has reported predominant distribution of AT₁ and AT₂ receptor subtypes in the endothelia of the blood vessels and the epithelia of the pancreatic ductal system of rat and mouse (393). The presence of the AT₂ receptor in the beta cells of the human islets of Langerhans, and in endothelial cells of the pancreatic vasculature, was demonstrated by Tahmasebi et al. (695).

D) FUNCTION. The pancreatic RAS appears to regulate secretion of pancreatic hormones. Elevated plasma sodium concentration resulted in depression of the binding affinity and capacity, whereas sodium depletion elevated both binding affinity and capacity of ANG II binding sites in rat pancreas (224). In a rat pancreatic acinar cell line (AR4–25), ANG II stimulated via AT₁ receptor a dose-dependent release of amylase (97).

In rat pancreas, chronic hypoxia caused a marked increase in angiotensinogen both at the protein and gene levels and an increase in AT₁β and AT₂ receptor mRNA when compared with that in the normoxic pancreas (91).

ANG II infusion induced a dose-dependent reduction in both whole pancreatic and islet blood flow in rats and points to a pivotal role of islet blood perfusion for an adequate insulin release (79). More recent studies suggest a role for ANG II-induced apoptosis of pancreatic acinar cells in pancreas fibrosis mediated by AT₁ receptors (763). This finding and the overall functional relevance of the tissue RAS in the pancreas awaits further study (396).

3. Stomach

A) ANGIOTENSINOGEN, ANG I, AND ANG II. Angiotensinogen mRNA was found in rat stomach (74). Ludtke et al. (422)
have reported phasic and/or tonic responses of human gastric smooth muscle to ANG II which often exceeded the maximum acetylcholine-induced activation.

**B) FUNCTION.** Cullen et al. (123) have found that captopril alleviated the systemic acidosis and the stress ulceration produced by canine hemorrhagic shock. Because during shock captopril enhanced blood flow to the small intestine, pancreas, liver, and spleen, but not to the stomach, the authors concluded that alleviating systemic acidosis was mediated through enhanced perfusion of other visceral organs.

**4. Intestine**

ANG pathways in the intestinal tract are represented in Figure 6.

**A) RENIN.** Renin gene expression was found in the intestine of human and mouse (636).

**B) ACE.** ACE has been localized in the brush border of human jejunum (678). In the rat, the highest ACE mRNA, ACE protein, and activity within the intestine were found in the brush-border membrane fraction in the proximal to midregion of the small intestine (186, 806). ACE activity was highly enriched on both the intestinal vascular plasma membrane and the intestinal brush border of the rat (764). ACE immunoreactivity was detected in human small intestine blood vessels (microvascular endothelium was strongly labeled) and the absorptive epithelial cells of intestinal mucosa, especially in microvilli and brush borders (62). Coste rousse et al. (110) reported that in the rat intestine, ACE mRNA levels and ACE activity were very high at birth and then decreased dramatically during the next 2 wk. ACE activity was reported also in the brush-border membrane from the small intestinal epithelium of the common grackle (679). ACE2 mRNA expression is ubiquitously found including the intestine (251); however, systematic expression analysis revealed a remarkable surface expression of ACE2 protein in enterocytes of the small intestine (251).

**C) ANGIOTENSINOGEN.** Angiotensinogen mRNA was detected in mesentery but not in small intestine of rat (74).

**D) ANGIOTENSIN RECEPTORS.** AT2 receptor mRNA and protein were characterized on cultured rat intestinal epithelial cells (661). The distribution of ANG II receptor was localized by Duggan et al. (171) via in vitro autoradiography. ANG II receptors were most abundant in the colon, followed by the ileum, duodenum, and jejunum. Within each segment of the intestine, specific ANG II binding sites were localized exclusively to the muscle layer. Sechi et al. (633) detected by autoradiography AT1 and to a lesser extent AT2 receptors in rat intestine. Specific binding was moderately abundant in the mucosa and the muscularis of both jejunum and ileum, whereas no binding was present in the submucosa and the serosa. In the colon, binding was significantly more abundant in the muscularis than in the mucosa. Hosoda et al. (288) could clearly demonstrate the distribution of AT1 immunopositive cells in enteric nerves and moderately in surface epithelial cells of guinea pig distal colon. Functional AT1 receptors were shown by Schinke et al. (618) in the rat ileum and duodenum.

**E) FUNCTION.** At low physiological doses, ANG II stimulates jejunal sodium and water absorption in adrenalectomized rats, whereas at a high dose the peptide inhibited absorption (400). However, the stimulation of jejunal water absorption was abolished in peripherally sympathectomized rats (400). In vitro study showed that mucosal additions of ANG II were more or less able to stimulate jejunal sodium and water absorption than serosal addition (399). Also, ANG III is able to stimulate rat jejunal fluid absorption. This effect can be blocked by guanethidine, phentolamine, and prazosin, suggesting that ANG III-increased intestinal absorption is secondary to the release of norepinephrine from sympathetic nerves in the jejunum (398). The same author has reported in another study that ANG II was able to enhance absorption in isolated rat jejunum in the presence of prazosin (399). This is contradictory to the hypothesis of involvement of the sympathetic nervous system in mediating ANG II.
stimulated fluid absorption. Cox et al. (116) have shown that ANG II, if applied to the basolateral surface, elicited increases in short-circuit current in preparations of rat jejunum and decreases in the descending colon. They have further shown that the response was not affected by α- or β-adrenoceptor antagonists but by a chloride channel blocker and that the increase/reduction in short-circuit current elicited by ANG II in jejunum/colon was inhibited by both piroxicam and indomethacin. Jin et al. (320) detected that in rat jejunum the low-dose ANG II-stimulated fluid and sodium absorption was blocked completely by the specific AT₂ receptor antagonist PD123319 but was unchanged by losartan. Conversely, high-dose ANG II inhibition of absorption was blocked by losartan but not by PD123319. The ANG II-mediated sodium and fluid absorption involved cGMP formation, and the high-dose inhibition via the AT₁ receptor is both negatively coupled to cAMP and increases jejunal PGE₂ production. A comparison between SHR rats and normotensive controls has shown that SHR possess an enhanced intestinal fluid absorption both in hypertensive adults and in normotensive young SHR (170). ANG II was also able to enhance water absorption and antagonized the secretory effect of vasoactive intestinal polypeptide (VIP) in the ileum similar to norepinephrine (49). The increased rat jejunal fluid absorption after extracellular fluid reduction is not affected by adrenectomy, but is abolished by nephrectomy, captopril, prazosin, and peripheral sympathectomy, suggesting that ANG II is generated after extracellular fluid reduction and increases jejunal fluid absorption by facilitating the release of norepinephrine from enteric sympathetic nerves (78, 399, 400). Serosal addition of ANG II produced a concentration-dependent short-circuit current in guinea pig distal colon (288). The authors reported that the ANG II evoked responses were mainly due to Cl⁻ secretion and mediated by the AT₁ receptor. Hatch et al. (263) reported that the enhanced K⁺ secretion in distal colon of rats with chronic renal failure was mediated by AT₁ receptors, since the secretion was reversed to an absorptive flux by losartan treatment, and rats with chronic renal failure exhibit twofold higher ANG II binding sites in distal colon compared with controls.

Yoshioka and co-workers (805, 806) detected that the peptidase activity of the rat brush-border membrane for specific peptide sequences can be inhibited by ACE inhibition. The authors suggested that intestinal brush-border membrane ACE functions as a digestive peptidase. This group also suggested (186) that ACE is an important intestinal dipeptidyl carboxypeptidase, participating in the digestion and assimilation of dietary peptides in rats (186). Suzuki et al. (690) detected that a diet high in proline is particularly effective in increasing intestinal ACE mRNA and activity in rats.

ANG II causes a contractile response in the rat ileum but not in the duodenum (617), pointing to a role in inducing peristaltic waves. In the ileum, the contraction is caused by the longitudinal smooth muscle and is mediated by the AT₁ receptor.

Jaszewski et al. (317) have reported that mucosal levels of ANG I and II were elevated in patients with Crohn’s colitis versus normal subjects and other forms of colitis and that mucosal levels of ANG I and ANG II correlated well with the degree of macroscopic inflammation in Crohn’s disease.

The RAS is highly activated during hypovolemic shock and has been suggested to be involved in the pathophysiology of the markedly deteriorated splanchic circulation seen in this condition. Aneman et al. (16) detected that the downregulation of mesenteric oxygenation and duodenal mucosal function during hypovolemia in pigs can be prevented by administration of enalaprilat, whereas guanethidine is ineffective in this respect. The authors concluded that this might be of clinical interest to support mesenteric perfusion and organ function in hypovolemia (16). Also, AT₁ receptor blockade ameliorated mesenteric and particularly jejunal mucosal hypoperfusion during hypovolemia in pigs (17). Furthermore, mortality in conjunction with acute hypovolemia and retransfusion could be completely avoided. Oldner et al. (517) reported that ANG II receptor antagonism increases gut oxygen delivery but fails to improve intestinal mucosal acidosis in porcine endotoxin shock. Tadros et al. (694) detected that ANG II appears to play a pivotal role in the burn- and endotoxin-induced intestinal ischemia and reperfusion injury in pigs, with subsequent increases in permeability and bacterial translocation and that administration of the ANG II receptor antagonist DuP753 after birth significantly reduces the extent of these events.

5. Colon

A) ANGIOTENSINOGEN. Angiotensinogen mRNA was detected in rat large intestine (74).

B) FUNCTION. It has been demonstrated that ANG II stimulated fluid transfer and sodium transport in segments of isolated rat proximal colon. Further investigation suggested that ANG II stimulated a coupled NaCl transport (145). In rat distal colon, ANG II can stimulate intestinal water transport via an electroneutral mechanism already at subpressor doses; this is a specific effect and in contrast to aldosterone which stimulates intestinal sodium and water transport by electrogenic mechanisms (402) involving the epithelial sodium channel (47).

G. Sensory Organs

1. Eye

The presence of a local RAS in the eye has been suggested by studies demonstrating the expression of all components of the system in ocular tissue. Wagner et al.
(752) have demonstrated that the mRNA of renin is expressed in the retinal epithelium, whereas angiotensinogen and ACE mRNA were found both in the retinal epithelium as well as in the neural retina of the eye. These data suggest that the machinery for ANG production is present locally and not only restricted to vascular structures in the eye. Evidence that the RAS is also expressed at the protein level has been provided by Sramek et al. (670, 671) who used immunohistochemistry against prorenin and angiotensinogen to describe prorenin in the ciliary body and angiotensinogen in the pigmented ciliary epithelium.

The question of whether the presence of the RAS components also leads to local production of ANG peptides has been answered by Danser et al. (131) who suggested that the ocular ANG concentrations are too high to be caused by blood-borne peptides.

A) Function. Whereas the presence of the components of the RAS has been well documented in the eye, little is known about its local function. Since ANG II receptors have been detected primarily on retinal blood vessels, it has been suggested that the ocular RAS may be involved in the regulation of ocular vascular tone. Apart from this aspect, the ocular RAS may be involved in the regulation of aqueous fluid since local application of ACE inhibitors influences aqueous fluid production. A recent report suggested that the ocular RAS may be relevant for maintaining normal secretory function of epithelial cells in the ciliary bodies (125). In this study, ANG II was defined as a secretagogue, leading to cell volume loss in cultured nonpigmented epithelial cells derived from human ciliary body. This process is antagonized by losartan, suggesting that ANG receptor blockade could be a new pharmacotherapeutic approach in the treatment of glaucoma. The pathophysiological role of the local RAS in the eye appears of special clinical importance since it has been linked to diabetic mellitus (682).

2. Cochlea

There are suggestions of an influence of ANG II on cochlear blood flow, which very well could be a secondary effect. Wright et al. (784) exposed guinea pigs to a 120-dB white noise for 30 min. The result was a fourfold elevation in plasma concentration of ANG II. A second experiment in the same study showed that an intra-arterial injection of ANG II (at concentrations comparable to those measured after noise exposure) increases the systemic blood pressure and cochlear blood flow. This finding suggests that elevated blood pressure, caused by noise exposure and mediated by ANG II, increases the cochlear blood flow, and it was suggested that this is due to a lack of a cochlear blood-pressure autoregulation in guinea pigs. Kawakami et al. (337) showed that cochlear blood flow of guinea pigs may have some autoregulation but was less than brain blood flow. Unlike this finding, Quirk et al. (563) have found a cochlea blood-flow autoregulation in rats mediated directly by ANG II. Intra-arterially infused ANG II produced an initial increase in cochlear blood flow that is followed by a slow steady return to baseline, despite sustained elevations in systemic blood pressure. Furthermore, a direct infusion of ANG II in the anterior inferior cerebellary artery of rats (563) results in significant reduction in cochlear blood flow without changes in systemic blood pressure. This effect can be abolished by ANG II receptor antagonism. Another study (107) investigated the role of ANG IV in the cochlear blood flow in guinea pigs. Anterior inferior cerebellary artery infusion of ANG IV increased in a dose-dependent manner the cochlear blood flow with little change in mean arterial blood pressure.

The role of ANG II in cochlear blood flow, however, appears to be species specific and is not completely understood. This notion is based on results from an investigation that has shown that ICV-administered ANG II leads to blood pressure elevation and that cochlear blood flow was more highly correlated with blood pressure in the guinea pig than in the rat in this setting (209).

A pathophysiological explanation of the controversy has been offered by Goldwin et al. (236). The discovery that during noise exposure insufficiencies in cochlear blood flow may cause temporary or permanent threshold shifts suggests that vasoactive substances including ANG II may mediate this effect. By pretreatment with ANG II receptor antagonists, both cochlear microcirculation and auditory sensitivity were measured during noise exposure. Results showed that pretreatment prevented this noise-induced microcirculatory ischemia and preserved auditory sensitivity at the low frequencies tested (236). In summary, the evidence for the existence of a local cochlear RAS is scarce and needs further analysis. Moreover, it is not at all clear whether local production of ANG II in the cochlea plays a significant role for the effects reported so far.
leukocytes (348). These authors have also detected ANG I staining, but the signal was less pronounced than for ANG II. Rat and mouse macrophages and monocytes contain ANG I and ANG II as well (156).

III. Angiotensin receptors. Shimada and Yazaki (647) demonstrated ANG II binding sites in human mononuclear leukocytes via autoradiography. ANG II binding sites were reported on guinea pig macrophages (708), which were found responsible for ANG II incorporation by these cells. Finally, Simon et al. (657) reported that ANG II binding to human mononuclear cell is not easily reversible and is poorly inhibited in a competitive manner, suggesting that endocytosis occurs.

Kim et al. (340) reported that ANG II increases monocyte binding to human as well as rabbit aortic endothelial cells, suggesting a role in arteriosclerosis.

B) Granulocytes. Human neutrophils possess the ability to convert ANG I to ANG II via cathepsin G, a lysosomal serine protease (349). Reilly et al. (571) reported a rapid conversion of ANG I to ANG II by human neutrophil cathepsin G and human skin mast cell chymase. And Wintroub et al. (780) demonstrated that angiotensinogen is also cleaved by cathepsin G, suggesting that a human granulocyte-angiotensin system does not require renin or ACE. Human polymorphonuclear leukocytes showed very little staining for ANG II (348), whereas ANG II can induce neutrophil chemoattractant release from human and bovine cultured endothelial cells (191) pointing at local regulation.

2. Spleen

A) Renin. With the use of RNase protection assays, renin mRNA was detected in rat spleen. Ekker et al. (179) have also found renin gene expression in rat and mouse spleen by primer-directed enzymatic amplification of cDNAs.

B) Angiotensinogen. Angiotensinogen mRNA was detected in rat spleen (74).

C) Angiotensin receptors. Binding sites for ANG II were localized in the red pulp of the spleen of rats and mice by quantitative autoradiography (86). ANG II binding sites were also localized on isolated rat spleen cells. Tsutsumi et al. (722) showed that the binding sites for ANG II are attributable to the AT1 receptor subtype. However, mouse spleen lymphocytes seem to posses both AT1 and AT1 receptor subtypes (368).

D) Function. ANG II significantly enhanced the [3H]thymidine incorporation into DNA of mouse splenocytes (368). The stimulatory effect was only blocked when losartan or PD123319 was added together with ANG II.

3. Thymus

A) Renin. Renin gene expression was found in rat and mouse thymus by primer-directed enzymatic amplification of cDNAs (179).

B) Angiotensin receptors. ANG II receptors were not detected in thymus sections from rats or mice, or on isolated rat thymocytes (86). But in newborn rats, Correa et al. (108) have detected AT1/AT2 receptors (15%/85%) in the trabecula. The binding sites were no longer detected in 4- and 8-wk-old rats.

I. Adipose Tissue

Apart from its presence in white adipose tissue where the local RAS may play a role in the pathogenesis of syndrome X, RAS components have also been detected in brown adipose tissue located adjacent to the adventitia of blood vessels, where it could provide an interface with the vascular RAS.

1. Renin

Renin mRNA was found in adipose tissue of obese humans (335). Renin activity was localized in interscapular brown adipose tissue, especially in adipocytes, which is not decreased after bilateral nephrectomy (643). Renin and all other components (angiotensinogen, renin binding protein, ACE, and AT1 receptors) that are necessary to form a local RAS were found in human preadipocytes (620), in human adipose tissue, and in cultured human adipocytes (183, 184).

2. ACE

ACE mRNA was found in both human subcutaneous and extraperitoneal adipose tissue, especially in adipocytes (328). Also, Karlsson et al. (335) have detected ACE mRNA in adipose tissue of obese humans. ACE synthesis at protein levels was detected in human preadipocytes (620).

3. Angiotensinogen, ANG I, and ANG II

Angiotensinogen mRNA was identified in periartrial and periaortic brown adipocytes and in fibroblast-like cells of periaortic connective tissue and mesentery of rat (73, 84). By Northern blot analysis, Karlsson et al. (335) demonstrated angiotensinogen gene expression in adipose tissue from adipose humans. Slices from interscapular brown adipose tissue are able to release immunoreactive ANG II peptides (643). Schling et al. (620) showed that ANG II was secreted both by undifferentiated human preadipocytes and immature adipocytes, and its production was significantly elevated in differentiated cells.

4. Angiotensin receptors

The receptor subtype that is present most abundantly in both white and brown adipose tissue is the AT1 receptor subtype. Mice adipose tissue expresses predominantly
AT$_{1A}$ subtypes (68). Crandall et al. (121) localized AT$_{1}$ receptor protein in rat epididymal, mesenteric, and retroperitoneal adipose tissue and human omental and subcutaneous adipose tissue. It is also notable that in human preadipocytes there was a high-affinity ANG II binding site of the AT$_{1}$ subtype (121). Rat white adipose tissue contained a greater number of ANG II binding sites than brown adipose tissue and both present the AT$_{1}$ subtype (83).

5. **Function**

ANG II plays a role in sympathetic nervous system-mediated thermogenesis. Cold exposure resulted in an increase in interscapular fat ANG II content, without concomitant changes in plasma components of the RAS, and led to an ANG II-mediated presynaptic facilitation of evoked norepinephrine overflow from rat interscapular fat during cold exposure that can be diminished by losartan (80). Cold exposure induced an increase of ANG II in brown adipose tissue without detectable changes in plasma components of the RAS and mediated facilitation of sympathetic neurotransmission (80, 81).

Treatment of cultured human preadipocytes with ANG II resulted in an AT$_{1}$ receptor-mediated increase of expression of the mRNA for cell cycle regulatory protein cyclin D1 (120). The role of ANG II in adipose cell maturation and growth was also supported by a study that showed the differences in angiotensinogen mRNA and protein content between young and adult rats (258). Angiotensinogen mRNA (protein) was approximately two-fold (3-fold) higher in adipocytes of young (8 wk) rats compared with old ones (26 wk). Interestingly, Brink et al. (60) have found that ANG II infusion to rats caused many systemic changes like decreasing skeletal muscle mass or reduced sympathetic neurotransmission (80, 81).

Safonova et al. (588) showed that angiotensinogen gene expression in adipocytes is activated by fatty acid levels in a dose-dependent manner. Jones et al. (327) showed that ANG II significantly increased triglyceride content and the activities of two key lipogenic enzymes (fatty acid synthase and glycerol-3-phosphate dehydrogenase) in 3T3-L1 and human adipose cells. Interestingly, this effect was mediated by the AT$_{2}$ receptor, in contrast to other publications that predominantly or exclusively detected AT$_{1}$ receptors (68, 121, 327). Body weight correlated positively with adipose angiotensinogen mRNA expression in humans (226, 732). Kouyama et al. (360) have shown that mice lacking AT$_{1}$ receptor have higher energy expenditure and thus show attenuation of diet-induced weight gain and adiposity. A role of angiotensinogen, derived from adipose tissue, in blood pressure regulation and adipose tissue volume has been postulated (438). Using angiotensinogen-deficient mice with reduced blood pressures as a background, the scientists generated transgenic mice with angiotensinogen expression restricted to adipose tissue. The animals showed circulating angiotensinogen, increased fat mass, normotension, and restored renal function (438). In addition, transgenic mice derived from wild-type mice and designed to overexpress angiotensinogen only in adipose tissue demonstrated elevated plasma angiotensinogen and developed hypertension (438). In another study, the authors could demonstrate by studying angiotensinogen-deficient and wild-type mice that angiotensinogen appears to be involved in the regulation of fat mass through a combination of decreased lipogenesis and increased locomotor activity that may be centrally mediated (439). ANG II has been shown to be able to induce leptin expression in adipocytes (344), and Cassis et al. (82) suggested that in contrast to systemic ANG II, only locally produced ANG II is able to elevate plasma leptin levels, since systemic ANG II also leads to sympathetic activation, which would diminish the effect of ANG II.

### III. PRENATAL DEVELOPMENT

Local ANG formation and tissue-specific effects of ANG peptides on growth and differentiation are thought to be extremely important for embryonic and fetal development. There appears to be a close interaction between plasma and tissue RAS in this situation.

1. **Renin**

Newborn rat plasma renin activity and plasma renin concentration are higher than adult levels (551, 755). In the rabbit fetus, plasma renin activity shows very low values but increases rapidly during the last 4 days of gestation and reaches a maximal level in the early postnatal period. Siegel and Fisher (651) have shown that furosemide-induced plasma renin activity was for the first time inducible at 106 days of gestation and increased with the gestational age in the fetal lamb and that the fetal lamb plasma aldosterone level did not increase in response to endogenous renin stimulation.

2. **ACE**

Rat somatic ACE is first identifiable at 18 days of gestation and increases thereafter until 2–6 wk postpartum (656, 753, 754). In addition, in the human fetal lung, ACE activity increased with gestation and reaches its mature activity earlier than those in the rat (656). Plasma ACE correlated with gestational age of fetal lambs and was associated with prepartum increases in plasma cortisol and blood pressure; by 2 wk of postnatal age, plasma ACE had decreased to the value seen in nonpregnant adult ewes. The maternal plasma ACE was similar at all
gestational stages (213). Also, in fetal rabbit, an increase of pulmonary ACE activity was detected from 22 days of gestation until term (672).

3. **Angiotensinogen**

The major portion of angiotensinogen mRNA in the embryo is most likely derived from the fetal liver, and the level in the whole rat embryo was increasing by day 13 and reached a plateau by day 17 (380). Furthermore, the authors suggested that the poly(A) tract of rat embryonic angiotensinogen mRNA may be longer than that of adult liver angiotensinogen mRNA.

4. **Angiotensin receptors**

The high abundance of AT$_2$ receptors in fetal tissue and in vitro studies suggesting the involvement of AT$_2$ receptors in mediating programmed cell death (797) and cell differentiation (680, 681) have lead to the hypothesis that ANG II, acting via the AT$_2$ receptor, is involved in aspects of organogenesis.

5. **Kidney**

A) **Mesonephros**. Renin is expressed very strongly from stage 13 in capillaries within glomeruli as well as in the wall of arteries in the interstitium and in the arterioles up to the aorta of the human embryonic mesonephros (630). ACE protein can be detected by immunohistochemistry at stage 14 in the apical membrane of the human mesonephric tubule cells. Angiotensinogen mRNA was detected by stage 12–13 in the proximal portion of the primitive tubules of the human mesonephros. The AT$_1$ receptor mRNA was detected in human mesonephric glomeruli, probably in mesangial cells, beginning at stage 12, and the AT$_2$ receptor mRNA was the component to be expressed first (stage 11) and was confined to the undifferentiated mesenchyme of the human mesonephros surrounding the preliminary tubules and glomeruli. In addition, both the mRNA and protein for AT$_1$ and AT$_2$ receptors were present in the ovine fetal mesonephros (69).

B) **Metanephros**. The distribution of renin mRNA in human metanepros appeared slightly different from that in the mesonephros as it had become confined to the juxtaglomerular apparatus at the vascular pole of the glomerulus and to dispersed cells of arteries located next to glomeruli. In human metanepros, ACE activity was detected in proximal convoluted tubules and collecting ducts in increasing amounts until birth (630). In the human metanepros, angiotensinogen mRNA showed only weak labeling macroscopically and was expressed in proximal tubules from 8 wk of age and throughout gestation. Hybridization signal for AT$_1$ receptor mRNA was observed in human metanepros glomeruli and in proximal tubular epithelium. Juxtaglomerular cells expressed AT$_1$ mRNA as soon as they differentiated. AT$_2$ receptor mRNA expression was highest in the human metanepros at 8–9 wk gestational age. It could be detected in undifferentiated mesenchyme, surrounding tubules and immature glomeruli. The AT$_2$ receptor mRNA signal in the human metanepros declined after 20 wk of gestation but remained detectable until birth. Also, both mRNA and protein for AT$_1$ and AT$_2$ receptors were present in the ovine fetal metanepros (69).

A recent study (504) has indicated a positive-feedback mechanism for renin generation in cultured developing rat metanepros. Renin mRNA was localized to developing tubules and uretal branches and glomeruli in the cultured explants. Also, both ANG II receptors and ANG II were detected in the rat cultured metanepros. After ANG II treatment, the authors detected an increase in renin activity that was prevented by AT$_1$ blockade, whereas AT$_2$ antagonism had no effect.

Maric et al. (434) have detected AT$_1$ and AT$_2$ receptor binding sites by radiolabeled binding assay and AT$_1$ and AT$_2$ receptor mRNA (RT-PCR) in isolated embryonic renomedullary interstitial cells from embryonic rat kidneys. The authors have demonstrated that ANG II acts as a potent mitogen to those cells in a time- and dose-dependent manner. This effect could be abolished with losartan but not with PD123319, suggesting that the mitogenic effect of ANG II is mediated through the AT$_1$ receptor. ANG II was also able to inhibit growth induced by basic fibroblast growth factor mediated by the AT$_2$ receptor (681). Gimonet et al. (228) have shown that both ANG II receptor subtypes are expressed early during nephrogenesis in fetal lamb but displayed specific spatial and temporal distribution during gestation. The level of AT$_2$ expression followed closely glomerular proliferation rate and disappeared after nephrogenesis completion. Also, in the differentiated epithelial cells of macula densa, AT$_2$ mRNA was detected. AT$_1$ receptor was predominant in mesangial cells of mature glomeruli but was also detected in cells invading the inferior cleft of S-shaped bodies and in medullar cells between tubules.

Mice lacking AT$_{1B}$ receptor are healthy, without an abnormal phenotype. In contrast, Agtr1a$^{-/-}$ Agtr1b$^{-/-}$ mice have diminished growth, vascular thickening within the kidney, and atrophy of the inner renal medulla (70). The complete AT$_1$ receptor knockout mice have no systemic response to infusions of ANG II, and the blood pressure is reduced substantially. Interestingly, after administration of an ACE inhibitor, their blood pressure increases paradoxically. This could be a result of disruption of AT$_2$ receptor signaling.

Miyazaki et al. (449) have shown that AT$_1$ receptor knockout mice do not develop renal pelvis or ureteral peristaltic movement. Comparing the Agtr1$^{-/-}$ neonatal mice with complete unilateral ureteral ligated wild-type neonates, the structural anomalies were qualitatively indistinguishable between the Agtr1$^{-/-}$ neonatal mice.
without surgical obstruction versus the wild-type mice with complete unilateral ureteral ligation. In both kidneys, the calyx was enlarged and the papilla was atrophic (449). Moreover, tubulointerstitial inflammation, e.g., macrophage infiltration and fibrosis, shows the same pattern. Thus the authors suggested that the abnormal kidney structure that develops in neonates during ANG inhibition is attributed largely to functional obstruction of the urinary tract caused by the defective development of peristaltic machinery (449). Interestingly, the irreversible renal abnormalities that are induced by ANG II type 1 receptor blockade or ACE inhibition were attenuated by simultaneous infusion of insulin-like growth factor I to rats at perinatal days 3–13 (499).

6. Adrenal gland

Renin was detected in the cortex of adrenal gland of mouse fetus, appearing as small patchy or granular reaction products in the perikaryon of a few cells. Later in the gestation, numerous tiny granules were found just below the cell membrane (358). The AT1 receptor mRNA could be detected in the neocortex of human adrenal gland at 8 wk and throughout the gestation (neocortex differentiates into the zona glomerulosa, fasciculata, and reticularis around the time of birth), whereas AT2 receptor mRNA is expressed earlier (5–6 wk) in a mass of condensed cells above the developing kidney, namely, the future adrenal fetal zone (630). The majority of ANG binding sites in primary cultures of both human fetal zone and neocortex cells were of the type 1 subtype (564). However, Breault et al. (58) detected by autoradiographic studies that ANG II receptors in the human fetal adrenals are mainly of the type 2, suggesting that culture conditions increase AT1 receptor expression.

In contrast to observations in rat fetus, the adrenal medulla of the human fetus does not express AT2 receptor mRNA (630). In the ovine fetal adrenal gland, Wintour et al. (779) could detect both mRNA and protein of the AT1 receptor in the zona glomerulosa and fasciculata of the cortex, but not in the medulla by 60 days of gestation. The mRNA of AT2 receptor was present in the same location (and absent in the medulla) from 40 to 130 days and declined to extremely low levels after 140 days (term is 145–150 days). The authors showed that the application of ANG II caused a significant down-regulation of the AT1 receptor mRNA expression, and the AT2 mRNA levels remained unchanged. The adult ovine adrenal expresses only AT1 receptor mRNA (zona glomerulosa and to a lesser extent in the zona fasciculata, but never in the medulla) (779).

Moritz et al. (464) showed a functional antagonistic effect of the ANG II receptors by aldosterone secretion by the midgestation ovine fetus. In this study, a short-term infusion of ACTH but not ANG II stimulated aldosterone production in the midgestation ovine fetus. However, plasma aldosterone could be stimulated by ANG II in the presence of a specific AT2 receptor blocker treatment, suggesting that this may be mediated by AT2 receptors.

The AT2 receptor shows high density in the medulla of the adult adrenal gland of rats and rabbits but is virtually absent from the medulla of the human and bovine adrenal glands.

7. Heart

Renin mRNA could be detected in a few cells of the human embryonic heart starting expression at stage 12 and became more abundant during the course of gestation, although the positive cells always remained isolated and preferentially located in the inner layer of the myocardium (630). ACE activity was first detected at stage 14 in the inner layer of the human embryonic heart wall by these authors. AT1 receptor mRNA was diffusely distributed over the human embryonic heart muscle, whereas AT2 receptor mRNA was detected earlier, expressed at a higher level, and located in the innermost layers of the myocardium. By quantitative autoradiography, ANG II receptors were first detected in the rat myocardium on embryonic day 14 and reached a maximum density within the first postnatal week (294).

Both ANG II receptor subtypes are involved in the regulation of type I and III collagen expression and structural protein accumulation in the developing rat heart, since type I collagen expression in cardiac tissue was reduced and type III collagen mRNA expression increased in both losartan and PD123319-treated rats (371).

A moderate density of binding to ACE was first detected in the rat cardiac vasculature and heart valves on embryonic day 19, whereas ACE was first detected in the rat myocardium on the day of birth, with the density of binding to ACE increasing during development (294). More recently, a pivotal role of ACE2 for normal heart development and function has been demonstrated for the ACE2 homolog ACER in Drosophila (119) and for ACE2 in mice (119).

8. Vasculature

During rat aortic development, AT1 expression was detected on gestational day 14, increased until embryonic day 16, after which levels were similar throughout postnatal development; conversely, AT2 mRNA first appeared at day 16, reached maximal levels between day 19 and neonatal day 1, and decreased thereafter (295). DNA synthesis rates decreased with aortic development, and AT1 receptor antagonism accelerates this developmentally regulated decrease in DNA synthesis, whereas AT2 receptor antagonism blunted this decrease. Yamada et al. (795) have also demonstrated that the expression of this receptor mediates decline in vascular DNA synthesis that
occurs at this stage of vascular development. The AT₂ receptor promotes also vascular differentiation and contributes to vasculogenesis. These authors could demonstrate that AT₂ knockout mice show lower mRNA levels for calponin in the aorta as the wild-type mouse aorta (795). Calponin is expressed in the late stage of vascular smooth muscle cell differentiation and correlates with expression of the AT₂ receptor. The authors have also demonstrated that the protein levels of calponin and high-molecular-weight caldesmon were lower in the aorta of AT₂ receptor knockout mice at 2 and 4 wk after birth.

9. Liver

In the human embryonic liver, hepatocytes show an extremely high level of angiotensinogen mRNA during formation from the foregut epithelium (630). On days 14–15, the rat embryonic liver had reached adult morphology, and during this period, angiotensinogen mRNA levels increased exponentially (380). Besides angiotensinogen, AT₁ receptor mRNA was also expressed in the human embryonic liver, but no expression of renin, ACE, and AT₂ receptor could be detected in the liver at any stage of development.

10. Brain

Immunostaining for ACE was observed in the developing nervous system and dermomyotome (630). Low levels of angiotensinogen mRNA were detected in the rat embryo head from days 17 to 19 (380). However, others have shown that angiotensinogen mRNA was more abundant in brain than in the liver in the early gestational life of rat embryo (soon after birth, brain angiotensinogen mRNA levels increased to a concentration 3-fold above fetal levels and liver angiotensinogen mRNA abundance increased 30-fold within 12 h of birth) (333). By immunostaining, angiotensinogen was first observed on rat embryonic day 18 in the choroid plexus and ependymal cells lining the third ventricle (474). During early postnatal development, a rapid progression of angiotensinogen staining appears in the astrocytes in the paraventricular nucleus, medial preoptic area, and ventromedial and arcuate hypothalamic nuclei. Sood et al. (663) have detected angiotensinogen immunoreactivity in the hindbrain and the spinal cord of rat fetuses. This immunoreactivity was associated with an active cell differentiation and cell growth. ANG II immunoreactivity was found in cells cultured from fetal rat brain of 20-day-old fetuses (770). These cells have been identified as neurons on the basis of morphological criteria. The AT₁ receptor could be visualized in parts of the developing brain (630).

Brain AT₂ mRNA was detected in many brain nuclei in the early stage of rat prenatal development, and there was strong but transient AT₂ mRNA expression in certain structures including the lateral hypothalamic neuroepithelium at day 13 of gestation; in the subthalamic and hypoglossus nuclei at day 15; in the pedunculopontine nucleus, cerebellum, motor facial nucleus, and the inferior olivary complex at day 17; in the thalamus, bed nucleus of the suprapontic decussation, interstitial nucleus of Cajal, nuclei of lateral lemniscus, locus ceruleus, and supragenual nucleus at day 19; and in the lateral septal and medial amygdaloid nuclei, medial geniculate body, and the superior colliculus at day 21. The substantia nigra and many telencephalic and medullary nuclei did not show AT₂ mRNA expression; however, AT₂ is expressed after birth in these regions (507). In nuclei involved in motor function and sensory integration, the AT₂ mRNA expression is high and persists until brain maturity (507). By autoradiography study, Tsutsumi and co-workers (721, 723) have detected AT₁ receptor binding sites in the nucleus of the solitary tract, subfornical organ, paraventricular nucleus, and choroid plexus of fetal rat brain; AT₂ binding sites were detected in the inferior olive, paratrigeminal and hypoglossal nuclei, in meninges, and cephalic soft tissues including cerebral arteries and high ACE concentrations in choroid plexus, subfornical organ, posterior pituitary, and cerebral arteries. Primary cultures of astrocytes obtained from fetal rats showed ANG II binding sites belonging only to the AT₁ subtype (56).

Distinct cells (probably from neural crest) around the neural groove and somites showed hybridization signal for AT₄ receptor. During later development, the sympathetic ganglion chain showed also a strong hybridization signal for the AT₂ receptor. Furthermore, all organs that contain components of neural crest origin were labeled at stages 14–16 (630).

11. Extraembryonic fetal tissues

A) Renin. Renin immunoreactivity was found in the human decidua and placenta (447). Renin mRNA and ACE immunoreactivity were detected in the chorion, renin mRNA in the cells of the chorionic mesenchyme, and ACE in the epithelium of the chorion (630). The presence of renin in human trophoblastic and amnioblastic cells was detected via immunostaining by Poisner et al. (552, 553). Paul et al. (532) demonstrated renin mRNA in the placenta. Renin mRNA was detectable in the human choriodecidua, whereas no renin mRNA was detected in the intertwin chorion, chorionic basal plate, placental villae, or amnion (299, 641). In the human choriodecidua, renin mRNA was localized in macrophages (319) and smooth muscle cells of veins and spiral arteries (460). Finally, the presence of renin protein was shown by Kalenga et al. (332). More recently, the newly high mRNA levels of the newly identified prorenin/renin receptor were detected in the placenta (488, 490).

B) ACE. ACE mRNA and activity in human or rat placenta were shown by several studies (332, 656, 793).
Human fetopathies were seen when ACE inhibitors were given around the 26th week of gestation. The major adverse effects in babies include the following: oligohydramnios, renal tubular dysgenesis, neonatal anuria, caecal arrest, and pulmonary hypoplasia, mild to severe intraterine growth retardation, persistent patent ductus arteriosus, and fetal or neonatal death (70).

C) ANGIOTENSINOGEN, ANG I, AND ANG II. Angiotensinogen mRNA was undetectable in any of the placental tissues and hydatidiform moles using Northern blotting (299). Angiotensinogen mRNA was demonstrated in human placenta and decidua using more sensitive detection methods (459, 532), and Lenz et al. (389, 390) found high angiotensinogen concentrations in homogenates of the human placenta. Furthermore, angiotensinogen was detected in the human placenta and decidua by immunohistochemistry (447). The human chorio-decidua was found to secrete ANG I in vitro (122).

In some tissues they appear to be regulated independently of the plasma RAS as can be concluded from experiments where nephrectomy (downregulating the formation of ANG II in the plasma) led to upregulation or no changes of locally formed ANG II such as in adrenal gland or brain. In other organ systems, there appears to be close cross-talk between the local and plasma RAS. Components of the system such as renin or angiotensinogen in the heart, for example, may be taken up from the circulation and stored locally to be available for local ANG synthesis and action. In some cases, specific independent actions have been assigned to RAS components, for example, in the testis where a role in fertility and sperm motility is discussed.

If there is a common denominator for the physiological role of these local systems, it is the maintenance of a balance or homeostasis at the tissue level between opposing effects mediated by the system such as growth promotion and inhibition, for example, in the heart and blood vessels. The dual actions of ANG II on its receptors can be considered as a basis for this balance. In addition, the possibility of alternative pathways including the use of different substrates such as AC-SDKP for ACE, different receptors such as AT4, and different processing products such as ANG-(1-7) can be responsible for mediating the regulatory and counterregulatory effects. If this balance is disturbed, for example, by overexpression of RAS components or inhibition of others, the RAS becomes a mediator of pathophysiological stimuli. In these regulatory processes, the plasma RAS takes up the role of an acute “response unit,” whereas tissue-based ANG II formation is more linked to subacute and chronic modulation. The concept of local or tissue-based RAS, therefore, should not be considered as an opposing or alternative but rather as a complimentary or integrated functional concept of ANG formation and function. There is clearly no basis for any controversy.

IV. SUMMARY

The local RAS exerts diverse actions in many organs. In some tissues they appear to be regulated independently of the plasma RAS as can be concluded from experiments where nephrectomy (downregulating the formation of ANG II in the plasma) led to upregulation or no changes of locally formed ANG II such as in adrenal gland or brain.


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Physiology of local renin-angiotensin systems


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