I. Introduction

Since its discovery 100 years ago, an impressive quantity of information about renin has been compiled. Notably, researchers have determined that the renin-driven renin-angiotensin-aldosterone system (RAAS) plays a major role not only in the homeostatic processes, such as blood pressure and fluid volume control, but also in the development and progression of fibrotic/hypertrophic diseases. We have learned to distinguish between the systemic RAAS,
which is mainly controlled by the production and release of renin from the kidneys into the circulation, and the more locally acting renin-angiotensin systems that are present in a variety of organs. This review predominantly focuses on kidney renin, and it is thus a follow-up of the milestone review published in *Physiological Reviews* 30 years ago (181). Since then, an influx of exciting new information about the renin gene and its regulation, the renin-secreting cells in the kidney and their regulation, and the activity of the renin-angiotensin cascade has accumulated and will be considered in this review. Among these new discoveries is the identification of the local renin-angiotensin systems, which were recently extensively reviewed in this journal (674) and will therefore not be the focus of the present review. Our review concentrates on the organization and transcriptional control of the renin gene and on the nature of renin-producing cells in the kidney. It further considers the regulation of renin secretion on the cellular level and its control by local or systemic factors. Finally, we consider the different biological signaling pathways and their biological effects initiated by renin activity.

**II. THE RENIN GENE**

The structure of the renin gene (*Ren-1*) has been determined for several different species, including sheep, rats, humans, dogs, and mice (13, 103, 245, 313, 341, 671). Sequence analysis has revealed high interspecies homology in the structural elements of the gene. More specifically, the mouse and rat renin genes possess nine exons and eight introns, while the human and sheep renin genes contain one additional exon (exon 5A) that encodes three additional amino acids. In both humans and mice, the renin gene was mapped to chromosome 1. Some inbred mouse strains are unique in having two renin genes. Mouse strains with a single renin gene possess *Ren-1C*, while mouse strains with two renin genes have *Ren-1D* and *Ren-2* (3, 228). The *Ren-1* and *Ren-2* genes are closely related and probably originated from a gene duplication that occurred about three million years ago (197, 352). The functional significance of this gene duplication remains unknown. *Ren-2* is strongly expressed in the submandibular gland (197, 229). In addition, the product of *Ren-1*, however, correlates best between the rat and human renin genes (214, 246). The product of *Ren-2* lacks key glycosylation sites and is consequently sorted to the constitutive secretory pathway (163) (see sect. IIIA). Originally, it was believed that mice with two renin genes should exhibit higher plasma renin levels. However, in contrast to this hypothesis, mouse strains expressing *Ren-2* have normal blood pressure (76, 578). A later systematic study demonstrated that plasma renin concentrations are actually not different between mouse strains with one or two renin genes (309).

A. Regulatory Noncoding Sequences of the Renin Gene

The 5'-flanking noncoding region plays a central role in regulating the renin gene in all species studied. This region is therefore considered as a classical promoter, i.e., a 5'-cis-acting regulatory element (Fig. 1). Most of the information on the function of the renin promoter has been obtained from cell culture models. The isolation of the clonal renin-producing cell line As4.1 from the kidney of simian virus 40 (SV40)-T antigen transgenic mice has provided an important tool for studying the cell-specific regulation of renin transcription (802a). The isolation of

![Fig. 1. Structural organization of the renin promoter. Schematic map of the 5'-flanking regulatory elements of the renin gene and the binding of the transcription factors. Double-headed arrows bind the conserved regulatory regions in mouse (mRen) and human (hRen) renin genes.](http://physrev.physiology.org/)
the native renin-expressing juxtaglomerular (JG) cells in primary cultures also represents a relevant model; however, it has two limitations. First, the expression of renin is turned off within several days in the culture, and second, JG cells usually represent only a minority of the isolated cells. These disadvantages of the primary JG cell cultures appear to have been overcome recently by a double-transgenic labeling of cells of renin lineage in vivo and their subsequent isolation and cultivation in vitro (681). However, this model is still awaiting a broader application to confirm its efficiency and reliability.

The function of renin gene regulatory sequences in vivo has been intensively studied during the last few years. Recently, a number of transgenic mice with various deletions in the renin promoter have been generated. Surprisingly, some in vivo results have deviated from the models predicted on the basis of the existing cell culture data (269a, 857, 989, 990). Some of these discrepancies may reflect species-specific differences; however, it also appears possible that the in vitro findings may not be completely relevant for the in vivo situation. In any case, there is still a great number of cell culture data on the function of the renin promoter that needs to be validated at the level of the whole organism. Therefore, in the following sections, which concern the molecular and cellular mechanisms of renin gene expression (sect. II, A–C), we have focused on the results from cells with endogenous renin production, followed by discussion of the in vivo data if available.

1. **The proximal renin promoter**

A 123-bp sequence from −117 to +6 in the mouse *Ren-1C* gene (all positions hereafter are relative to the transcription starting site) was originally defined as a “proximal renin promoter” (690). This sequence was found to be necessary for the maximal cell-specific expression of mouse and human renin genes (83, 690). The proximal promoter is highly homologous in human, mouse, and rat renin genes and contains a perfectly conserved TATA box. Further upstream, the homology is interrupted by a −500-bp insertion that is only present in mouse renin genes (106). Currently, the term *proximal renin promoter* refers to the 5′-regulatory sequences located roughly downstream to position −200, since a row of important cis-regulatory elements has subsequently been identified in this region.

The general transcription factor binding site pattern of the proximal renin promoter suggests that this region is important for the basal activity of the renin gene, for the transcriptional regulation by cAMP and nuclear receptors and for the control of renin gene expression during ontogenesis.

**A) PROXIMAL PROMOTER CIS-ACTING ELEMENTS TARGETED BY cAMP.** Renin is a cAMP-inducible gene. The presence of a functional cAMP response element (CRE) is a characteristic feature of the renin promoter in all species tested (83, 264, 442, 620, 663, 794, 975). A proximal CRE sequence is located at −226 to −219 in the human renin gene (975). However, only the 3′ half of this motif is evolutionarily conserved, which makes its role in the regulation of the renin gene uncertain. There is substantial evidence suggesting that several CRE-unrelated proximal promoter sequences are also involved in the cAMP-dependent regulation of the renin gene. A Pit-1 transcription factor binding site at −77 to −67 in the human renin promoter is necessary for stimulation of renin transcription by cAMP (265, 833, 834). The homologous sequence in the mouse *Ren-1C* gene, which spans −72 to −50, was also shown to be important for transcriptional activity. This DNA motif is bound by the homeodomain proteins HOX and PBX (135, 665, 669). The HOX/PBX core binding sequence was recently found to be indispensable for the basal renin expression level in the kidney in vivo (269a, 857).

Interestingly, the very same sequence is targeted by the retinoblastoma gene product (RB) to stimulate the *Ren-1C* promoter activity in human embryonic cells, which do not however express endogenous renin (852). Therefore, the role of RB in the cell-specific control of renin transcription remains to be further elucidated. The nuclear receptor LXR-α also mediates the cAMP effect via a motif called CNRE (cAMP and negative regulatory elements). The CNRE is located at position −128 to −115 in the human renin gene and at −611 to −599 in the mouse *Ren-1D* gene (356, 357, 850, 851). This sequence displays little homology to either the CRE or the classical DR4 LXR-responsive element (DR4/LXRE). However, the partial deletion of the proximal promoter LXR-α binding motif affected neither the basal expression nor the regulation of the renin gene in vivo, thus contradicting the physiological significance of the CNRE (see also sect. nCl) (857).

**B) FURTHER CIS-ACTING ELEMENTS IN THE PROXIMAL RENIN PROMOTER.** The human renin gene was recently identified as the first gene regulated by the transcription factor peroxisome proliferator-activated receptor-γ (PPAR-γ), which occurs through a nontypical Pal3 (palindrome with a 3-bp spacer) binding site located at −148 to −134 (881). Although the renin Pal3 site resides in an evolutionarily conserved promoter region, we found that mouse and rat renin Pal3 elements are transcriptionally silent (882). The critical bases of the Pal3 motif that confer full inducibility by PPAR-γ were mapped to the 5′-repeat and to the last base of the spacer (882). The human renin Pal3 sequence is unique in selectively binding PPAR-γ, which permits the maximal agonist-dependent stimulation of transcription, particularly at low cellular levels of PPAR-γ. Thus the human renin gene appears to be more sensitive to PPAR-γ than its murine orthologs.
The nuclear effector of the Notch signaling pathway (CBF1) bound to a sequence in the proximal promoter (−210 to −191, −679 to −660, and −185 to −166 in human, mouse, and rat renin genes, respectively) was recently identified to be a partner of the HOX/PBX transcription factors (665, 669). Both the Notch system and the HOX proteins are important during embryonic development; more specifically, they are believed to participate in regulating the tissue-specific expression of the renin gene (494, 559, 665). In addition, the developmentally active Ets transcription factors have also been reported to interact with sequences within the proximal renin promoter (665).

Another important regulatory sequence lies within the proximal promoter of the mouse Ren-1C gene, just upstream of the HOX/PBX-binding site (667). The deletion of this DNA-fragment (−197 to −70) resulted in an almost complete loss of renin transcriptional activity. At least two transcription factor families, namely, NFI and Sp1/Sp3, bind to this region of the proximal promoter (667).

2. Renin gene enhancers

In addition to the proximal promoter region, the renin gene is controlled by two known 5′-flanking enhancer elements. An enhancer is typically defined as a regulatory DNA sequence that strongly induces transcription in an orientation and position-independent manner.

a) Renal enhancer. A 242-bp enhancer element was identified at −2866 to −2625 in the mouse Ren-1C gene. This sequence was shown to stimulate renin transcriptional activity up to 100-fold in the renin-producing cell line As4.1 (690). Since the As4.1 cells are of renal origin, the Ren-1C enhancer is called the “renal” or “kidney” enhancer (690, 802a). Emerging evidence suggests that the mouse renal enhancer directs the on/off switching rather than the transactivation rate of the renin gene in the single cell in vitro (601). Such a variegation model of renin expression control is feasible because the renin-producing cells are “recruited” during chronic stimulation in vivo (see sect. uF). However, additional studies are necessary to confirm or reject this concept.

The renal enhancer is also present in the human renin promoter, although at a more distal position (about −12 kb) (966). The human and mouse kidney enhancers are highly homologous in their 202-bp distal portions, while the similarity in their 40-bp proximal fragments (m40) is only 45% (794). The heterology in the m40 sequence explains the lower transactivating capacity of the human enhancer compared with the mouse enhancer (379; see below).

The role of the renal enhancer in the regulation of renin expression in vivo is still not completely understood. Studies of transgenic mice expressing human renin from an artificial chromosome have demonstrated that the renal enhancer of the human renin gene is dispensable for the stimulation of renin gene expression by angiotensin converting enzyme (ACE) inhibition (989). In contrast, deletion of the renal enhancer of the endogenous renin gene in mice has revealed that it is necessary for the full activation of renin transcription by the combination of a low-salt diet and the ACE inhibitor (7, 562). On the other hand, both the human and mouse renal enhancers appear to be critical for the basal transcription of renin, both in vitro and in vivo (7, 989).

b) cis-Acting elements in the renal enhancer. The downstream enhancer portion of the renin gene contains a CRE and a more proximal E-box, which bind the CREB/CREM and USF1/USF2 transcription factors, respectively (442, 663). These binding sites are critical for the function of the renal enhancer, since a mutation of either one results in an almost complete loss of its activity in As4.1 cells (663). Moreover, CRE mediates the stimulatory effect of the cAMP/PKA cascade on renin expression. Thus cAMP signaling has at least two targets in the renin gene regulatory sequences, the nonconsensus CRE site located in the proximal promoter (see above), and a canonical CRE located in the renal enhancer. It is still not understood whether the distinct cAMP-targeted cis-acting elements are redundant, thus underscoring the role of cAMP in the transcriptional control of the renin gene, or whether each has specific functions.

Two directly repeated TGACCT sequences are located downstream and adjacent to the E-box (379, 796). This hexamer repeat represents the DNA binding site for the superfamily of nuclear receptors and is generally known as the hormone responsive element (HRE) (29). Consistently, the nuclear receptors retinoic acid receptor/retinoid X receptor (RAR/RXR), PPAR-γ, and the orphan receptor Ear2 were found to bind to this enhancer element (521, 796, 882). It is important to note that the human renin HRE differs from the mouse sequence both in the spacer between the hexamer repeats and in the proximal hexamer repeat (794). While the deviations in the spacer are not critical for the transcriptional activity of the enhancer, the substitution from A to G in the proximal hexamer of the HRE is responsible for the lower transactivating capacity of the human kidney renin enhancer (379). In the mouse renin kidney enhancer, the downstream TGACCT motif is overlapped by an NF-Y transcription factor binding site (794, 795). It has been suggested that NF-Y prevents the binding of transcriptional regulators to the hexamer motif, thus inhibiting the enhancer’s activity (795).

There are at least seven important transcription factor binding sites in the more distal enhancer fragment. One of these motifs binds the Wilms’ tumor suppressor, WT1, which inhibits renin transcription (826). The other six motifs are bound by the NFI and Sp1/Sp3 transcriptional regulators (664). Notably, the same transcription
factors bind to the proximal renin promoter (667). It remains to be clarified whether these multiple renin promoter binding sites are redundant or have different functions. The mutation of either the enhancer or the proximal NFI/Spi1/Spi3 binding sequences almost completely eliminates the renin promoter activity, thus indicating a crucial role for these transcription factors in the control of renin gene expression (664, 667).

C. Chorionic Enhancer. A second enhancer was identified in the human renin 5'-flanking region between −5777 and −5552 (263). This enhancer delivered an ~60-fold induction of the human renin promoter activity in chorionic cells and, therefore, is known as the "chorionic" enhancer (263). However, the chorionic enhancer is not evolutionarily conserved and was consequently found to be transcriptionally silent in transgenic mice (990).

3. Intron I regulatory sequences

In addition to the promoter sequences, regulatory DNA elements have been identified in intron I of the human and rat renin genes (262, 919). Intron I has been shown to exert a silencing effect on the renin gene expression. Closer characterization of the rat intronic silencer sequence in vitro has led to the identification of two positive and five distinct negative cis-acting elements (918).

B. Regulation of Renin mRNA Stability

In addition to the tight transcriptional control described above, renin gene expression is also regulated at the posttranscriptional level (147, 499, 809). In general, the mechanisms that stabilize or degrade mRNAs are not completely understood. For renin mRNA, it is known that its stability is controlled by mRNA binding proteins, which interact with the 196-bp-long 3'-untranslated region (UTR) of renin mRNA. Multiple mRNA binding proteins have been shown to bind to the renin mRNA 3'-UTR (6, 811). HuR, CP1/hnRNP E1 [poly(C)-binding protein-1], nucleolin, dynamin, and YB-1 are thought to stabilize renin mRNA. The HADHB [hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase (tri-functional protein)-β-subunit] protein is believed to destabilize renin mRNA, while the effects of the MINT-homologous protein and the hnRNP K protein on renin mRNA stability are unknown. Thus, in addition to the transcriptional mechanisms, posttranscriptional modifications of renin mRNA are also important for the control of renin gene expression. However, it is currently difficult to evaluate to what quantitative extent each of these two mechanisms contribute to the regulation of renin mRNA abundance. In fact, the single cell produces more transcripts than the accumulated RNA steady-state level, demonstrating the existence of constitutive RNA degradation mechanisms (reviewed recently in Ref. 358). Therefore, it has been proposed that changes in the mRNA stability provide a faster response of renin gene expression to regulatory signals (602).

C. Control of Renin Gene Expression at the Cellular Level

Altogether, our knowledge of the intracellular mechanisms that regulate renin transcription suggests that the cAMP/PKA/CREB and calcium/PKC cascades are employed by physiological cues, whereas STATs, NFκB, and members of the nuclear receptor superfamily probably only become relevant in pathological situations. Similar to the renin gene regulatory sequences, most of the information on the cellular control of renin transcription comes from cell culture models. Consequently, not much is known about the relevance of the in vitro identified molecular mechanisms for the regulation of renin gene expression in the whole organism. Therefore, transgenic animals with selective and cell-specific gene inactivation are expected to deliver novel information on this issue.

1. cAMP and protein kinase A

The second messenger cAMP is produced by adenylyl cyclases, which in turn are regulated by extracellular stimuli through G protein-coupled receptors. cAMP exerts its effects on transcription by activating protein kinase A (PKA) (497). cAMP binds to the two regulatory subunits of PKA to release two catalytic subunits from the inactive PKA tetramer complex. The free catalytic subunits (referred to as activated PKA) translocate to the nucleus and phosphorylate transcription factors of the cAMP response element binding protein/activating transcription factor (CREB/ATF) family. These transcription factors are constitutively bound to CRE in the regulatory sequences of cAMP-driven genes and are transactivated by the PKA-induced phosphorylation (596). cAMP signaling is believed to be the central stimulatory pathway in renin gene transcription (442, 663). cAMP mediates the stimulation of renin expression by catecholamines/sympathetic activation and prostaglandins (146, 401, 473). In addition, the cAMP/PKA cascade essentially determines the basal transcription rate of the renin gene (663). CREB is a prototype representative of the CREB/ATF transcription factor family that mediates cAMP signaling to the renin gene (265, 442, 497, 663, 975). CREB was found to bind to the renin CRE sequences identified in the proximal promoter and in the kidney enhancer. CREM (cAMP response element modulator) and possibly ATF1, which are close relatives of CREB, also interact with the CRE enhancer (442, 663). Notably, all three of these proteins share the conserved target sequence for PKA.
Studies in transgenic animals have unambiguously confirmed the central role of cAMP in the physiological control of basal renin gene expression. Chen et al. (146) generated a transgenic mouse line with a specific deletion of the $G_{\alpha} \alpha$ protein in renin-producing cells (146). $G_{\alpha} \alpha$ is an essential subunit of the stimulatory G protein that couples adenyl cyclase with activating receptors such as the IP, EP4, and the $\beta$-adrenergic receptors. These receptors, in turn, mediate the effects of prostacyclin, prostaglandin $E_2$, and catecholamines on renin expression (241, 423). The local knockout of $G_{\alpha} \alpha$ in renin-producing cells has been shown to sharply diminish renal renin mRNA levels and plasma renin concentrations (PRC) (146). Unfortunately, the G protein receptor agonist-induced stimulation of renin gene expression was not tested in the JG-specific $G_{\alpha} \alpha$ knockout mice. Thus the role of cAMP signaling in the physiological regulation of renin transcription in vivo remains to be definitely confirmed.

The binding of the nuclear factor LXR-$\alpha$ as a monomer to the CNRE proximal promoter motif was also reported to mediate the cAMP-dependent stimulation of renin transcription (356, 357, 850, 851). PKA has been suggested to phosphorylate LXR-$\alpha$ at two potential sites within its ligand binding domain (LBD) (426). Additionally, the COOH-terminal AF-2 transactivation domain in the LXR-$\alpha$ molecule was found to be necessary for responsiveness to cAMP (850). The renin gene in LXR-$\alpha$ $--/-$ mice is insensitive to adrenergic stimulation (599); however, the CNRE sequence is dispensable for the regulation of renin expression in vivo (857). Therefore, the mechanisms of regulation of renin transcription by LXR-$\alpha$ need to be further investigated.

cAMP also increases the stability of renin mRNA in cultured cells (see sect. $\alpha$B) (147, 499, 811). In support of this observation, the adenyl cyclase activator forskolin increased binding of the stabilizing factors HuR and CP1 to the 3′-UTR of human renin mRNA (6).

2. Calcium and protein kinase C

In contrast to the stimulatory function of cAMP, cytosolic calcium and/or protein kinase C (PKC) are involved in the suppression of renin expression (188, 606). This unusual role for calcium/PKC is termed the “calcium paradox” of renin production. Hormones such as angiotensin (ANG) II and endothelins inhibit renin gene expression and secretion by increasing the cytosolic calcium and/or activation of PKC (293, 485, 606, 727, 728). However, the mechanism by which calcium and/or PKC inhibits renin synthesis remains, to a large extent, unknown. Calcium was recently found to inhibit the transcription of the human renin gene by interacting with a calcium response element in the promoter region (243). ANG II targets the proximal 2.8 kb (606), while endothelin-1 targets the enhancer element of the mouse Ren-1C promoter (737). In addition, like cAMP, calcium influences renin mRNA stability (see sect. $\alpha$B). The RNA-binding protein dynamin has been suggested to destabilize renin mRNA in response to increased intracellular calcium (443).

3. STAT and NF$\kappa$B transcription factors

Inflammatory cytokines, such as interleukin-1$\beta$ (IL-1$\beta$) and tumor necrosis factor-$\alpha$ (TNF-$\alpha$), were found to be potent inhibitors of renin production (52, 399, 668). Cytokines appear to be relevant as factors that regulate the renin expression during acute or chronic inflammation. The cardiovascular response to inflammation includes severe hypotension or septic shock (when hypotension is accompanied by organ failure), resulting from the dysregulation of vasoconstrictor and vasodilator systems, whereby the activity of the latter becomes dominant (92–94, 345). Since renin is the limiting factor for the generation of ANG II, which, in turn, is a potent systemic vasoconstrictor, it has been proposed that the cytokine-mediated inhibition of renin expression is an important mechanism in the pathogenesis of hypotension/septic shock upon inflammation (52, 691). Accordingly, mice treated with bacterial lipopolysaccharide (LPS) in an acute model of sepsis express significantly less renin mRNA in the kidneys, compared with their controls (52).

Neither cAMP nor the calcium signaling pathways are currently known to be involved in the intracellular mechanism of action of the cytokines on renin gene expression. At least two transcription factors have been reported to mediate the suppression of renin transcription by cytokines in cultured renin-producing cells.

A) STATS. STAT (signal transducers and activators of transcription) transcription factors play key roles in interferon- and hematopoietic growth factor-dependent stimulation of transcription (179, 947). The inflammatory cytokine oncostatin M was first found to suppress renin transcription through STAT5 (52). More recently, the cytokines IL-1$\beta$ and interleukin-6 were demonstrated to inhibit renin promoter activity via the extracellular signal-regulated kinases (ERKs) and STAT3 (522, 668).

B) NF$\kappa$B. We found that the transcription factor NF$\kappa$B is involved in the inhibition of renin gene expression through the cytokine TNF-$\alpha$ (880, 883, 884). NF$\kappa$B interacts with CRE in the renal enhancer without directly binding to the DNA. TNF-$\alpha$ employs at least two mechanisms to suppress renin gene expression. TNF-$\alpha$ inhibits the transactivating capacity of the NF$\kappa$B-p65 subunit and attenuates the binding of CREB to the CRE motif of the renal enhancer. The latter mode of trans-inhibition is particularly interesting because it could represent one of the underlying enhancer-dependent mechanisms for switching on/off the transcription, according to the variegation model of regulation of the renin gene. Moreover, a similar mechanism is utilized by the vitamin D$_3$.
The retinoic acid receptor—the nuclear receptors in the renal renin enhancer (29). The presence of a HRE sequence that is generally targeted by the nuclear receptors in the renal enhancer (29). The retinoic acid receptor-α and the RXR-α were identified as members of the protein complex bound to the renin enhancer HRE, and treatment with retinoic acid stimulated renin promoter activity in an enhancer-dependent manner (796). RXR-α also binds to a proximal cis-acting element in the human renin promoter (−148 to −134), known as Pal3 (881). Although the role of this motif in the regulation of renin gene expression by vitamin A is still unknown, Pal3 was found to be targeted by another member of the nuclear receptor superfamily, PPAR-γ (881). Interestingly, PPAR-γ stimulates human renin transcription through the proximal promoter Pal3 sequence, while the human renin enhancer HRE seems to be dispensable for this effect (881). PPAR-γ most likely targets murine renin genes through the HRE enhancer sequence. Together with their different protein-binding properties (881; see also sect. iiA, i and 2), the PPAR-γ-targeted sites of the renin gene (HRE and Pal3) are important examples for the single transcription factor-binding nonredundant cis-acting elements, which may mediate species-specific regulation. Therefore, we are currently generating several transgenic mouse strains to study the mechanisms of the PPAR-γ-driven renin transcription in vivo.

Unexpectedly, the vitamin D$_3$ receptor (VDR) does not interact with the renin HRE enhancer site. Instead, the ligand-activated VDR represses the renin transcription by heterodimerizing with CREB and preventing it from binding to the renin enhancer CRE (980). Vitamin D$_3$ is considered to be a (patho)physiologically relevant inhibitor of renin gene expression (516, 980). Accordingly, VDR-deficient mice have increased renin production, which has led to increased water intake, hypertension, and cardiac hypertrophy (516).

D. Sites of Renin Expression

It is generally accepted that the active renin found in the circulation of mammals almost exclusively originates from the kidneys. However, significant amounts of plasma (pro)renin are produced in the submandibular salivary gland of some mouse strains. As previously mentioned, these mouse strains possess two renin genes, of which Ren-2 is highly expressed in the salivary glands (3, 197, 229). This renin gene duplication is specific to the mouse, and its functional significance remains unknown. Notably, even in nephrectomized and sialectomized animals, substantial pro-renin levels can be measured (405), and the origin of the residual pro-renin remains unclear.

In addition to systemic renin, there are a number of extrarenal tissues that express renin as a part of the local or tissue-specific RAASs. In these tissues, renin is expressed at a much lower level than in the kidney; its effects are therefore believed to be auto- and/or paracrine (293). Renin is also produced locally in organs that exhibit a blood-tissue barrier, such as the brain or the testes (190, 208, 214, 255, 670). In these organs, the local renin operates independently of the systemic RAAS. On the other hand, in organs such as the heart and the large arterial vessels, significant amounts of renin are taken up from the plasma to support the paracrine activity of the locally produced enzyme (627, 685).

Within the kidney, renin is predominantly produced by the JG cells. Interestingly, renin is, to a lesser extent, also synthesized in the renal proximal tubule, connecting tubule and collecting duct, and it is believed to be involved in the local regulation of salt reabsorption and cellular proliferation by ANG II (448, 858, 860). In particular, the expression and regulation of (pro)renin in the connecting tubule and collecting duct have attracted attention over the last few years (729). Renin expression in this localization may be important under conditions of high ANG II states like diabetes (417, 688) and renovascular hypertension (702).

Renin mRNA was detected in the organs of the gastrointestinal tract, the immune system, and the female reproductive system, as well as in the adrenal gland, adipose tissue, eye, and skin (214, 229, 246, 270, 383, 420, 433, 735, 803, 842, 929). The local effects of tissue renin are cell and tissue specific. These local RAASs were recently assessed in several excellent reviews (448, 512, 674).

E. Development of Renal Renin Expression

During nephrogenesis, renin expression changes in a uniform manner in mammals (115, 200, 204, 272, 273, 275, 457, 458, 584, 694, 803), as recently illustrated in a detailed three-dimensional view of renin expression during the development of mouse kidneys (756). Renin first appears in the undifferentiated metanephric blastema of embryonic kidneys (789) before vessel formation has begun, suggesting that renin cells originate within the metanephric kidney from renin progenitor cells rather than from an extrarenal source. Once vascularization of the kidney has begun (around embryonic day 14 in mice and rats), renin-producing cells are exclusively distributed along the walls of the arteriolar vessels (133, 584, 726). Renin in association with the vasculature is first present on day 15 of
embryonic development, as it begins to be expressed in distinct cells scattered throughout the distal part of the evolving vascular tree. From this initial expression site, renin begins to expand bidirectionally into larger renal vessels, in which renin expression is not continuous and does not resolve into a clearly predictable expression pattern. As nephrogenesis proceeds, the arterial tree continues to mature, and the expression of renin shifts from larger vessels to newly developed afferent arterioles. Subsequently, the renin completely disappears from the larger vessels, becomes more and more restricted to the afferent arterioles, and is finally localized at the classic juxtaglomerular position. Recent data suggest that the acquisition and maintenance of the renin cell identity is mediated by cAMP and histone acetylation at the CRE of the renin gene (681). The embryonic cells expressing renin can also give rise to other cell types, and as a consequence, inhibition of the development of renin-producing cells leads to developmental defects of the whole kidney (682). Interestingly, cells that have switched off renin expression during kidney development can be reactivated to produce renin in adult life (788) (see sect. II F).

Among the afferent arterioles, renin expression is first observed in the afferent arterioles of the juxtamedullary zone of the kidney, and it later increasingly appears in the midcortical and finally in the cortical zones (Fig. 2). The functional relevance of this characteristic shift in renin expression during metanephrogenesis is not entirely clear. It has been speculated that renin-producing cells might guide the development of intrarenal blood vessels, since in rat kidneys the development of a new arterial branch is preceded by the appearance of renin expression at the point of branching (720). In contrast, presumptive bifurcations of the developing arteries in the mouse kidney are free of renin, while robust expression is detected at the mature branch points. The absence of renin in the branching arterial tips, as well as intense expression in the more established vessels, also suggests that renin expression is not a prerequisite for the formation of the vascular tree in the mouse kidney (756, 844).

The cellular mechanisms responsible for the characteristic developmental changes in intrarenal renin expression are not well understood. One may assume that the spatiotemporal appearance of direct regulators of renin gene expression may coincide with the initiation or termination of renin expression during nephrogenesis. Some studies have shown that the distribution of intrarenal renin expression parallels the development of sympathetic innervation of the kidney (703), which reflects the centrifugal pattern of renin expression and renal vasculature. In addition, the expression of the transforming growth factor (TGF)-β type II receptor shifts in a manner similar to that of renin during renal development (520), suggesting that TGF-β might also be involved in triggering developmental renin expression. The evidence for a distinct role of cell-to-cell communication via gap junctions in the distribution of renin-producing cells has recently been obtained. Renin-producing cells at all developmental stages express the gap junction protein connexin40 (492). Mice lacking connexin40 display a normal pattern of renin expression in the larger vessels, but they are unable to maintain renin-producing cells in the afferent arterioles (493). Instead, renin-producing cells in the mature kidneys are located outside the vessel walls in the periglomerular interstitium.

The proper regulation of developmental renin expression requires switching on and off the relevant transcription factors. It has been reported that the putative mRNA splicing protein Zis may be of relevance for the development of renin-expressing cells (419). However, its mode of action in renin-producing cells has not yet been elucidated in detail. The renin gene promoter contains

FIG. 2. Renin during embryonic kidney development. Reconstruction of the α-smooth muscle actin immunoreactive structures (red) and of the renin immunoreactive areas (green) in the developing mouse kidney. The distribution of renin is shown in whole organ reconstructions (A–C) and in developing branches of arcuate arteries (D–F) on embryonic day 16 (A and C), postnatal day 1 (B and E), and in the adult kidney (C and F). The light green color indicates glomeruli.
consensus binding motifs for many transcription factors (see sect. II A), two of which might be considered favorite candidates. One is the Hox transcription factor, generally known to be relevant for development, while the second is the CRE factor, CREB (663). The deletion of both Hox binding motifs (665, 666, 669) and CRE sites (7, 146, 442, 666, 853) has been shown to strongly reduce the basal renin promoter activity and to blunt its stimulation in vitro. Preliminary evidence suggests that the interruption of the cAMP pathway in vivo can nearly prevent renin expression during kidney development (624).

F. Recruitment of Renin Expression in the Adult Kidney

Plasma renin is produced and secreted by the renal JG cells (see sect. II). These epithelial-like cells are located in the wall of the afferent arteriole in close vicinity to the glomerulus (293). JG cells comprise a very small cell population in the kidney. Notably, the number of renin-producing cells in the afferent arteriole is variable, such that chronic stimulation of renin synthesis increases their number, and vice versa. These changes in the number of renin-producing cells in the afferent arteriole are based on the unique ability of the smooth muscle(-like) cells of the afferent arteriole to acquire a secretory phenotype via a reversible metaplastic transformation. Thus, in states of increased renin production such as during impaired renal perfusion, salt depletion, or inhibition of ANG II formation, the larger portions of the afferent arteriole proximal to the JG region become renin-positive (114, 146, 788). The cellular mechanisms underlying the recruitment of renin-producing cells in the vessel walls of adult kidneys are not well understood. It was suggested that vascular smooth muscle cells may switch into renin producers, and vice versa, by a metaplastic transformation (114). An elegant study has recently provided evidence that not all smooth muscle-like cells in the renal vasculature can undergo such a switch and that the cells that can do so are genetically programmed (788).

III. THE JUXTAGLOMERULAR CELL

Several aspects render the renin-producing JG cells somewhat mysterious. The first aspect is the yet unexplained special position of the cells in the media layer of afferent arterioles at the entrance into the glomerular capillary network. The second unusual feature is the cuboid, epithelial-like form, which coined the term juxtaglomerular epithelioid (JGE) cells. Third, the cells contain numerous, sometimes large, dense-core secretory vesicles, but they very rarely show signs of exocytotic events. Finally, the regulation of secretion appears rather unusual, as calcium inhibits rather than facilitates renin secretion.

A. Processing and Secretion of Renin in Juxtaglomerular Cells

Renin shares the functions both of a hormone and of a protease. Therefore, on one hand, renin is intracellularly processed and glycosylated like a typical secretory protein. On the other hand, it is intracellularly processed and packed into vesicles like a typical lysosomal protein, which finally undergoes regulated secretion.

1. Renin secretory vesicles

Renin is initially synthesized as a pre-pro-renin protein. After cleavage of the prosequence, the pro-renin is directed to the cis-Golgi cisternae. From there, pro-renin can be immediately secreted by the constitutive pathway or sorted to the dense-core secretory granules for regulated exocytosis (Fig. 3). The correct sorting of the prorenin to the regulated secretory pathway depends on the presence of a paired basic amino acid site in the pro-renin molecule, which serves as a protease processing site for granule-attached prohormone convertases (85). In addition, a decrease in the pH of the early renin granules may facilitate the aggregation of the granule core. Accordingly, the proteogranules often contain paracrystalline cores (862). In mice with two renin genes (Ren-1D and Ren-2), the deletion of Ren-1D, but not Ren-2, is associated with a complete absence of the dense-core secretory granules in JG cells (163, 607), suggesting that the ability to form the dense-core secretory vesicles depends on the structural characteristics of the Ren-1 protein. These characteristics may be related to the glycosylation of the protein because renin originating from the Ren-1D gene has three N-linked glycosylation sites, while the Ren-2-derived renin has none. Renin glycosylation may be necessary not only for renin trafficking to the dense-core secretory vesicles (607), but also for the mannose-6-phosphate receptor-mediated uptake of renin or pro-renin in other tissues, such as the heart (685, 900).

In addition to pro-renin, the proteogranules contain proteases such as prohormone convertases (85, 95) and cathepsin B (579, 625), which can cleave off the 43-amino acid NH2-terminal prosensegment. These proteases require an acidic pH for optimal activity. The autoactivation of the proteases from their own pro-forms also depends on a low pH of 4–6. The low pH of renin secretory vesicles allows them to be stained with fluorescent dyes like quinacrine (17, 122, 687). Quinacrine staining of renin-containing vesicles has been used for dynamic visualization of live cells to trace the exocytosis of renin-containing vesicles (687).
As in other cells, the early secretory granules in renin-producing cells can take up extracellular protein by endocytosis, including horseradish peroxidase, which is targeted to the early granules without transit through the Golgi apparatus (865). The interaction of circulating pro-renin with tissue renin receptors may lead to proteolytic or nonproteolytic activation, as well as local generation of angiotensin and the activation of second messenger pathways (see sect. VI).

The ability to segregate renin into dense-core secretory granules for regulated exocytosis seems to be specific to native JG cells in situ. After isolation, JG cells rapidly lose the ability to direct renin into this pathway, whereas the chronic stimulation of the renin system increases circulating levels of both pro-renin and renin (885). Even in renal juxtaglomerular cells in situ, only 25% of synthesized renin is directed to the dense-core secretory granules, while 75% is constitutively secreted as pro-renin (699). This source, together with the pro-renin originating from extrarenal sources, accounts for the observation that the proform accounts for 80–90% of the total renin in human circulation (824).

Although the rates of renal renin synthesis and secretion can vary significantly within and between individuals, the density of renin granules in secretory cells is surprisingly constant. For example, in rats under normal laboratory conditions, one renin granule is found, on average, in a cell volume of 5 $\mu$m$^3$ (715, 813). A very similar density was observed during chronic salt depletion when the overall renin synthesis and secretion are strongly stimulated. Instead of elevating the number of granules per cell, the total number of renin-producing cells increases during salt depletion. The gross morphology of renin secretory granules of newly recruited JG cells is similar to that of the granules in the control situation. The mouse and rat renin granules have average volumes of 0.63 (400) and 0.35 $\mu$m$^3$ (715), respectively. Therefore, the long-term regulation of renin synthesis and secretion appears to be mediated by altering the number of renin-producing cells, rather than by modifying the processes involved in the control of renin synthesis and secretion in the individual cell (see sect. II).

2. Mechanisms of renin secretion

A substantial amount of evidence indicates that the regulated release of renin proceeds via traditional exocytotic mechanisms. Thus the spontaneous release of renin at the cellular level is discontinuous in the renin content per discharge, which corresponds to the calculated renin content of one secretory granule (813). Furthermore, the acute stimulation of renin secretion in vivo, for example, by renal artery constriction, leads to a marked decrease in the number of renin storage granules (715), while the average size of the individual granules does not change. Elegant in vitro studies using two-photon microscopy...
have confirmed the rapid disappearance of renin storage granules upon the acute stimulation of secretion (687). Recent support for exocytosis as the pathway for renin release is derived from capacitance measurements of single renin-producing cells of the kidney. The average capacitance of a mouse JG cell is ~3.1 pF (235), yielding a calculated cell volume of ~500 fl (500 μm³). Rat cells have a smaller capacitance of 2.6 pF, reflecting a somewhat smaller cell volume (235, 239).

The other conditions known to stimulate renin secretion in vivo and in vitro (see below) lead to as much as a 15% increase in capacitance, reflecting the insertion of the vesicle membrane into the plasma membrane (235, 236, 239). It has been estimated from whole cell patch-clamp studies that the number of granules that are released within minutes after maximal stimulation constitutes ~20% of the total vesicle number. This might indicate the presence of a pool of immediately available renin storage granules, the existence of which has also been postulated from studies of isolated glomeruli (812) and isolated perfused kidneys (486).

The activation of renin secretion is accompanied by the formation of deep plasma membrane invaginations. These invaginations likely result from the addition of membrane material, obtained from many secretory granules, to the cell surface and to already-fused secretory granules (compound exocytosis) (715, 734). These invaginations may explain why the discharge of the renin secretory vesicles does not necessarily require movement of the vesicles to the cell perimeter (687).

The molecular events that drive and regulate the exocytosis of the renin storage vesicles remain unknown. A single finding in this context is that the renal renin-producing cells express the calcium-dependent activator protein for secretion (CAPS). This family of proteins is specifically involved in the secretory process of large dense-core vesicles but not small clear secretory vesicles (822).

**B. Electrical Properties of Juxtaglomerular Cells**

The electrophysiology of renin-producing JG cells has been investigated in some detail using electrodes or patch-clamp techniques on single JG cells or JG cells in situ in isolated glomeruli with attached afferent arterioles (recently reviewed in Refs. 236, 238; see also Table 1).

Vasoconstrictors, which are known to suppress renin release, depolarize JG cells in situ (96, 97). In addition, an increase in the extracellular K⁺ concentration or an inhibition of K⁺ channels not only depolarizes JG cells, but also suppresses renin release (158, 269, 480, 487). On the other hand, the membrane hyperpolarization induced by K⁺ channel activation or Cl⁻ channel inhibition is accompanied by a stimulated renin secretion (227, 239, 396, 403, 615). Furthermore, the pharmacological inhibition of the Na⁺–K⁺–2Cl⁻ cotransport activity using furosemide leads to the hyperpolarization of single JG cells while concomitantly stimulating renin exocytosis (128). Both effects of furosemide appeared to depend on the Na⁺–K⁺–2Cl⁻ co-transporter (NKCC1) isoform, since neither was observed in NKCC1-deficient mice (128). Taken together, there is good reason to assume that changes of the membrane potential of JG cells are associated with renin release, such that depolarization is accompanied by suppressed renin exocytosis, while hyperpolarization is accompanied by stimulated renin exocytosis. Despite the clear association between both parameters, it is not clear whether changes in the membrane potential directly and causally control renin release.

The depolarization of JG cells in response to vasoconstrictors, such as ANG II, probably results from the inhibition of inwardly rectifying K⁺ channels (Kir) by a G protein-mediated process (483). Besides the Kir (235, 483, 507), there is electrophysiological evidence of at least two additional potassium (K⁺) conductances in JG cells. The first conductance is associated with a delayed outwardly rectifying K⁺ current (235, 239, 483) that has been identified as the BKₐCa zero splice variant; additionally, this conductance is activated by cAMP and results in hyperpolarization (239). The second conductance is associated with the K ATP channels (732). In addition to potassium channels, there is good evidence for the presence of NKCC1 and Ca²⁺-activated chloride channels in the plasma membrane of JG cells (128, 418, 483). Ca²⁺-activated Cl⁻ channels might contribute to membrane depo-

### Table 1. Expression and function of ion channels in JG cells

<table>
<thead>
<tr>
<th>Channel</th>
<th>Regulation in JG Cells</th>
<th>Function</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inward rectifier K⁺ channels; Kᵦ</td>
<td>Inhibition by angiotensin II</td>
<td>Depolarization by angiotensin II</td>
<td>4, 92, 229, 468</td>
</tr>
<tr>
<td>Ca²⁺-activated K⁺ channels; BKₐCa zero variant</td>
<td>Activation by cAMP</td>
<td>Hyperpolarization</td>
<td>4, 68, 229, 233</td>
</tr>
<tr>
<td>ATP-sensitive K⁺ channels; K ATP</td>
<td>Pharmacological activation: hyperpolarization, stimulation of renin secretion</td>
<td>Pharmacological blockade: hyperpolarization, stimulation of renin secretion</td>
<td>381, 706</td>
</tr>
<tr>
<td>Na⁺–K⁺–2Cl⁻ cotransporter1; NKCC1</td>
<td></td>
<td></td>
<td>123</td>
</tr>
<tr>
<td>Ca²⁺-activated Cl⁻ channels</td>
<td>Activation by Ca²⁺</td>
<td>Depolarization, inhibition of renin secretion</td>
<td>388, 468</td>
</tr>
<tr>
<td>Voltage-dependent Ca²⁺ channels, L type</td>
<td>Activation by depolarization</td>
<td>Calcium influx at positive membrane potentials</td>
<td>233</td>
</tr>
</tbody>
</table>
larization in response to vasoconstrictors. Thus Ca\(^{2+}\) mobilization by vasoconstrictors could stimulate Ca\(^{2+}\)-activated Cl\(^{-}\) channels, Cl\(^{-}\) efflux, and membrane depolarization.

JG cells are strongly electrically coupled to the neighboring cells of the afferent arteriole (96, 483, 732) (see sect. mM). Interestingly, their resting membrane potential changes in situ from \(-60\) to \(-80\) mV in nonpressurized arterioles (96, 483, 732) to approximately \(-40\) mV in pressurized arterioles (539). Since the depolarization of JG cells is accompanied by the suppression of renin release, the depolarization in response to an increase in perfusion pressure might directly or indirectly contribute to the known pressure-dependent inhibition of renin secretion.

Since Ca\(^{2+}\) inhibits renin secretion, it is clearly possible that the potential-operated Ca\(^{2+}\) channels mediate an influence of membrane potential on renin secretion. However, functional studies on the role of potential-operated Ca\(^{2+}\) channels have produced conflicting results (159, 487, 764; for a detailed discussion, see Ref. 779). A recent study by Friis et al. (239) might shed some light on this controversy; these authors provided evidence for the presence of L-type Ca\(^{2+}\) channel mRNA, protein, and functional activity in JG cells. However, because these channels are only activated at very positive membrane potentials (239), they are unlikely to be operational in JG cells under physiological conditions. These data might explain why several studies have failed to detect a direct functional role of the L-type Ca\(^{2+}\) channels in the regulation of renin release (779).

Finally, there is good, albeit indirect, evidence in JG cells for the store-operated Ca\(^{2+}\) entry triggered by the filling state of the internal Ca\(^{2+}\) stores (283, 483, 973). The identity of the channels underlying this Ca\(^{2+}\) influx is currently unclear. It will be interesting to investigate the role of ORAI proteins ("calcium release-activated calcium modulator,” pore-forming subunit of a calcium release activated calcium channel), as well as TRPC channels, in the regulation of Ca\(^{2+}\) influx and renin release in JG cells, since these channels contribute to or mediate store-operated Ca\(^{2+}\) entry in other cell types (817).

C. Intercellular Contacts of Juxtaglomerular Cells

The different cell types that form the juxtaglomerular apparatus (JGA) are abundantly coupled via gap junctions (861, 864). The intense intercellular connection creates a functional syncytium within this multilayered complex and may contribute to the regulation of the pregglomerular arteriolar tone, the glomerular filtration rate (GFR), and the activity of the renin system. Within the JGA, JG cells form a number of gap junctions among themselves (95), as well as with their neighboring endothelial, smooth muscle, or extraglomerular mesangial cells (583, 859, 861, 864).

Gap junctions are made up of connexin (Cx) proteins, which comprise a family of at least 20 protein isoforms (818). It was recently shown that JG cells predominantly express connexin 40 (Cx40) and, to a lesser extent, Cx37 and Cx43 (31, 296, 364, 983). The coexpression of Cx40 with Cx37 and Cx43 is specific to renin cells in the typical juxtaglomerular position, as renin-producing cells situated outside the classic juxtaglomerular position express only Cx40. Thus fetal renin-producing cells, which are still located in the wall of the larger renal arteries (756), and renin cells recruited upstream along the afferent arteriole by chronic challenge of the renin system showed only a Cx40-mediated coupling. For this reason, Cx40 may be considered as a fundamental feature of renin-producing cells (492). In addition to being found in renin-producing cells, Cx40 has been detected as one of the four main connexins (Cx37, Cx40, Cx43, Cx45) expressed in the wall of renal vessels (191, 290), where it is found almost exclusively in the endothelium.

Our knowledge of Cx40 expression in renin-producing cells has been mainly obtained from morphological analyses, and the functional relevance has not yet been extensively studied. However, one could imagine that the Cx40-mediated junctions between JG cells and the adjacent endothelium or the extraglomerular mesangium might provide a suitable pathway for the propagation of blood-borne or macula densa-derived signals involved in the control of the renin system.

Mice harboring a genetically disrupted Cx40 gene have been useful tools for investigating the functional importance of this connexin. Cx40 knockout mice are hypertensive (185) and show a high renin status (465, 922). An obvious explanation for the unusual combination of high renin levels with high blood pressure is that the transmission of inhibitory signals to JG cells does not work in the absence of Cx40. In fact, studies have shown that the classic negative-feedback mechanisms of renin secretion and renin gene expression exerted by ANG II or intrarenal blood pressure are disturbed in Cx40-deficient mice (465, 922); these data support the model of Cx40-dependent transmission of inhibitory signals to JG cells. Experimental unilateral renal stenosis in Cx40 knockout mice did not lead to the increase in blood pressure or elevated plasma renin levels that would typically be observed in wild-type mice. Furthermore, the typical increase in renin mRNA in the kidney with impaired perfusion is absent in Cx40 knockout kidneys. However, whether the special importance of Cx40 in JG cells is related to the unique properties of this connexin remained uncertain and was subsequently addressed by Cx40 knock-in Cx45 (Cx40KI45) mice. In these mice, the coding region for Cx40 was replaced by Cx45 under the control of the native Cx40 promoter (12). Cx45 exhibits a lower
In addition to these functional consequences, the structure and positioning of renin-producing cells in the JG position is strikingly altered in Cx40 knockout mice (493). Renin-producing cells are typically located in the media layer of the afferent arteriole, close to the vascular pole of the glomerulus. Although renin-producing cells display a high degree of plasticity during renal development (756) and chronic stimulation of the renin system (see sect. II, E and F), they are normally integrated into the wall of the arterial vessels. Ectopic renin expression in cells outside the medial layer of arteries has been described as a rare event that occurs, if at all, in the extraglomerular mesangium (457). In kidneys lacking Cx40, renin-producing cells are absent from the wall of the afferent arterioles. Instead, they are found around the wall of the afferent arterioles, extending into the region of the extraglomerular mesangium and the periglomerular interstitium. These ectopic renin-producing cells do not show the typical epithelioid appearance, instead exhibiting a more irregular mesenchymal-like shape. Interestingly, the expression of renin in the developing kidney of Cx40 knockout mice is restricted to the walls of the larger arteries, and it does not appear in the interstitial space until the wave of renin expression shifting along the arterial tree during nephogenesis finally reaches the glomerulus (493). One may, therefore, speculate that Cx40 is required for the correct “homing” of renin-producing cells in the JGA. It remains to be determined whether the Cx40-mediated contacts act as transmembrane channels that conduct unidentified signals relevant for the differentiation and proliferation of JG cells or whether they function as scaffolds that enable the correct assembly of other proteins relevant to the positioning of renin-producing cells in the classic location (216).

Apart from Cx40, JG cells also express Cx37 and Cx43 (492). Cx37 expression is not coregulated with Cx40 when the renin system is stimulated (465), but it cannot be demonstrated in renin-producing cells in the absence of Cx40. In contrast to mice lacking Cx40, Cx37-deficient mice are normotensive (807), suggesting that Cx37 might be less relevant for JG cell function than Cx40. In fact, our preliminary information suggests that the renin system works quite normally in the Cx37-deficient mice (926).

The role of Cx43 in the function of JG cells is at present less clear. Mice in which Cx43 is replaced by Cx32 (Cx43KI32 mice) displayed markedly lower renin levels than the wild-type controls (295). Notably, the reduction of renal perfusion pressure by unilateral renal artery stenosis, which normally leads to an increase in renin secretion and synthesis in the affected kidney and decreases renin content in the contralateral intact kidney, did not affect the renin expression in Cx43KI32 mice. This observation might indicate that, in addition to Cx40, Cx43 might also be critically involved in the control of the renin system. In summary, the intercellular signaling via con-
nexins appears to be an important determinant for the control of the renin system and for normal localization of JG cells.

D. Intracellular Signals Controlling Renin Release

At the cellular level, the numerous systemic and local factors that regulate renin release merge into three intracellular second messenger systems: the cAMP, cGMP, and calcium (Ca$^{2+}$) pathways (Fig. 4).

1. cAMP

Undoubtedly, cAMP is the central intracellular stimulator of renin release. This conclusion is supported by several lines of indirect and direct evidence. For instance, the activation of β-adrenoreceptors, which are known to enhance adenylyl cyclase activity, stimulates the renin secretion in a broad range of experimental in vitro models, including single JG cells as well as in vivo (54, 235, 423, 484, 903, 944). Moreover, the renin release in response to renal nerve stimulation is mediated by the β1/β2-adrenoreceptor knockout mice possess low plasma renin concentrations (PRC) (434). [Note that PRC is measured as the rate of ANG I generation in the presence of excess renin substrate. Plasma renin activity (PRA) is measured as the generation of ANG I without the addition of exogenous renin substrate.] In addition to catecholamines, other hormones, such as the prostaglandins E$_2$ and L$_2$ (prostacyclin) (402), dopamine (476), calcitonin gene-related peptide (482), pituitary adenylyl cyclase activating polypeptide (PACAP) (322), and adrenomedullin (397), stimulate the renin release from isolated JG cells and increase the intracellular cAMP concentrations.

The stimulation of adenylyl cyclase activity in response to activation of G protein-coupled receptors is mediated via the stimulatory G protein G$\alpha$. In line with the stimulatory role of G$\alpha$ and cAMP for renin release, the PRC is markedly reduced in mice carrying a conditional deletion of G$\alpha$ in JG cells (146). Notably, PRC is not stimulated in the G$\alpha$ KO mice in response to catecholamines, inhibition of the macula densa mechanism, or a drop in blood pressure, underscoring the central role of the cAMP pathway in the regulation of renin release (146).

Additionally, the direct activation of adenylyl cyclase activity by the diterpen forskolin strongly increases cAMP levels and renin release in isolated JG cells (283, 484), as well as in the renin-producing cell line As4.1 (283, 442). Finally, a direct link between cytosolic cAMP levels and renin release was demonstrated by the fact that mem-

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**Fig. 4.** Intracellular signaling pathways controlling the renin exocytosis. ANP, atrial natriuretic peptide; AC5/6, adenylyl cyclases 5/6; cGK, protein kinase G; DAG, diacylglycerol; GC-A, guanylate cyclase A (particulate guanylate cyclase); GP, GTP-binding protein; IP$_3$, inositol 1,4,5-trisphosphate; NO, nitric oxide; PDE3a, cAMP-phosphodiesterase 3a; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; sGC, soluble guanylate cyclase.
brane-permeable cAMP analogs and direct intracellular applications of cAMP via a patch pipette rapidly stimulate renin secretion (235).

Intracellular cAMP levels are determined not only by cAMP formation, but also by cAMP hydrolysis to 5’-AMP through the activity of cAMP phosphodiesterases (PDEs). The inhibition of cAMP degradation, either by nonselective inhibition of PDE activity using xanthine derivatives, such as 3-isobutyl-1-methylxanthine (IBMX) or theophylline, or by selective blockers of the isozymes PDE-1, PDE-3, and PDE-4, stimulates renin release (154, 156, 157, 479). Moreover, strong expression of PDE-3 and PDE-4 was confirmed in single JG cells and in the JG cell line As4.1 (237, 442). PDE-3 activity is regulated by cGMP, with an increase in the cytosolic cGMP inhibiting PDE-3 activity, which eventually results in elevated cAMP levels (57). Via this pathway, cGMP can indirectly stimulate renin secretion, which is of major physiological importance for the influence of NO on renin release (see below).

In conclusion, numerous studies using different approaches have clearly indicated that cAMP is the central stimulator of renin release. However, little is known about the mechanisms through which cAMP triggers the exocytosis of renin storage vesicles. While it has been shown that the stimulation of renin release by cAMP involves a PKA-dependent step (237, 477), the phosphorylation targets of PKA in JG cells are currently mostly unknown. It has recently been reported that JG cells express functional cAMP-sensitive BKCa channels and that the activation of these channels by cAMP results in hyperpolarization of the cells (239). As discussed earlier, the hyperpolarization of JG cells appears to favor exocytosis, suggesting that the activation of BKCa channels by cAMP might lead to enhanced renin exocytosis. However, the pharmacological inhibition of the BKCa channels does not attenuate the stimulation of renin release in response to cAMP (239). Therefore, a future challenge will be to identify the downstream signaling events that mediate the stimulation of renin exocytosis by the cAMP/PKA pathway.

2. Calcium

While cAMP is the main stimulator of renin release, the free cytosolic Ca2+ concentration is considered as the primary inhibitor. Notably, an increase in the intracellular Ca2+ concentration initiates and supports exocytosis in all other secretory cells, excluding parathyroid gland cells (165). Accordingly, the unusual effect of Ca2+ in the renin-secreting JG cells has been termed as the “calcium paradox” of renin release.

Early indirect evidence for an inhibitory influence of calcium on renin secretion was obtained from the observation that classical vasoconstrictors suppress renin release (293, 779). Thus vasoconstrictors such as angiotensin II, endothelin, vasopressin, and norepinephrine not only increase the Ca2+ influx or intracellular Ca2+ levels in JG cells (283, 369, 484, 485), but they also inhibit the renin release in a Ca2+-dependent fashion (587, 843). Other measures that result in an elevation of the cytosolic Ca2+ concentration also suppress renin release. For instance, thapsigargin, which blocks the sarcoplasmic reticulum Ca2+-ATPases and induces the store-operated Ca2+ influx, elevates the intracellular Ca2+ concentration in JG cells and suppresses renin release from isolated perfused kidneys and cultured JG cells (283, 781, 973). Conversely, the chelation of intracellular Ca2+ using 1,2-bis(2-aminophenoxy)ethane-N,N,N’,N’-tetraacetic acid (BAPTA) stimulates renin release from isolated juxtaglomerular cells (587, 654, 655).

Extracellular and intracellular calcium concentrations are positively correlated in JG cells (483). Therefore, it was correctly predicted that lowering the extracellular Ca2+ concentration would stimulate renin secretion in several in vitro models (293, 488, 863) and that the infusion of Ca2+ would suppress renin secretion from the nonfiltering kidneys of dogs in vivo (940). In addition to the parallel changes in the extra- and intracellular Ca2+ concentrations, the Ca2+-sensing receptor, which also controls the release of the parathyroid hormone in a Ca2+-dependent fashion, may participate in the regulation of renin release by the extracellular Ca2+ concentration (653).

Similar to the stimulation of renin release by cAMP, the downstream signaling pathways that mediate the suppression of renin exocytosis in response to an elevation in the cytosolic Ca2+ concentration are still under investigation. There is evidence to suggest that the suppression of renin release by Ca2+ involves the following: 1) Ca2+/calmodulin-dependent processes, such as the activation of myosin light chain kinase; 2) the modulation of ion channel activities in the plasma membrane or in the membrane of the renin-containing granule; or 3) the activation of PKC.

Since these studies have been the subject of previous reviews (293, 473, 779), we focus on a further downstream target of Ca2+, namely, the Ca2+-inhibited adenylyl cyclases AC5 and AC6 (283, 655). Both AC5 and AC6 are inhibited by physiological increases in the intracellular calcium concentrations and are therefore well positioned to link the inhibitory calcium pathway to the stimulatory cAMP pathway in an inverse manner in JG cells. In fact, the expression of AC5 and AC6 mRNA has been found in JG cells, while protein expression in these cells has so far been demonstrated only for AC5 (283, 654, 935). Moreover, an inverse relationship between the cytosolic Ca2+ and cAMP levels has been found in primary cultures of JG cells. While the increase in intracellular Ca2+ that occurred in response to endothelin-1, ANG II, or thapsigargin...
gin was paralleled by a reduction of the intracellular cAMP levels and renin release (283), the intracellular Ca\(^{2+}\) chelator BAPTA-AM had the opposite effect (654, 655). Since the inverse relationship between intracellular Ca\(^{2+}\) and renin release was prevented by clamping the intracellular cAMP levels or by the inhibition of adenyl cyclase activity, these data suggest that the modulation of cAMP by Ca\(^{2+}\) is an important determinant of the inverse relation between calcium and renin release (283, 655). The functional role of AC inhibition by Ca\(^{2+}\) has been further corroborated in gene knockdown studies (283) and in pharmacological studies using the adenyl cyclase inhibitor NKY-80, which shows preferential binding to AC5 compared with the non-calcium-inhibited isoforms AC2 or AC3 (648, 654).

Although the inhibition of cAMP formation by Ca\(^{2+}\) provides a possible explanation for the calcium paradox, future work should attempt to verify the functional roles of AC5 and AC6 in more complex models and in vivo, since the regulation of adenyl cyclase activity does not appear to completely explain the calcium paradox at the whole organ level (283). Moreover, further studies are needed to integrate other factors known to contribute to the Ca\(^{2+}\)-dependent inhibition of renin release, such as the activation of the myosin light-chain kinase or the modulation of channel activity, into this model of Ca\(^{2+}\)-inhibited adenyl cyclase mediation of the calcium paradox.

3. cGMP

In contrast to cAMP and Ca\(^{2+}\), which have been unequivocally shown to either stimulate or suppress renin release, the effects of cGMP are more complex, given that it can both stimulate and inhibit renin secretion.

On one hand, the application of membrane-permeable cGMP analogs at high concentrations suppresses renin release in a variety of models, including dispersed JG cells (276, 332, 475, 477, 633, 771). On the other hand, unmodified cGMP stimulates renin exocytosis upon direct injection into single JG cells (237). These contradictory effects can be best explained by the different affinities of each type of cGMP for intracellular target molecules. While cAMP-degrading phosphodiesterases such as PDE-3 are inhibited more efficiently by cGMP than by the stable cGMP analogs, the latter show a much higher affinity for cGMP-activated kinases (cGKs) (108). Therefore, these data indicate that cGMP can either stimulate renin release via the inhibition of PDEs or suppress renin secretion via cGK-dependent processes.

In fact, the cGMP-inhibited PDE-3 plays a mediator role in the stimulation of renin release by NO. Although the data are not unequivocal, NO has been shown to stimulate renin release in a variety of in vitro models, as well as in vivo (489). This stimulatory effect of NO is mimicked by the pharmacological inhibition of PDE-3, a PDE isoform that hydrolyzes cAMP (156, 157, 237, 479). Moreover, NO donors increase both intracellular cAMP concentration and renin secretion in primary cultures of JG cells, similar to the effects of PDE-3 inhibitors (477). These data suggest the existence of a cascade of signaling events connecting the cGMP and cAMP pathways in JG cells, where NO stimulates the formation of cGMP, which in turn suppresses PDE-3 activity and cAMP hydrolysis. Since cAMP is the central stimulator of renin release, the resulting intracellular accumulation of cAMP eventually enhances renin exocytosis. In fact, this interplay between the cGMP and cAMP pathways has been confirmed in vivo (59) in isolated perfused kidneys (479) and in patch-clamp experiments using single JG cells, in which cGMP stimulated renin exocytosis in a PDE3-, cAMP-, and PKA-dependent manner (237).

In addition to PDE-3, JG cells express cGK. cGKI is found in the cytosol, while cGKII is associated with renin granules (253, 254). Stable cGMP analogs suppress renin release in isolated perfused kidneys and in microdissected glomeruli with attached afferent arterioles (254, 927). As mentioned above, the stable cGMP analogs show a high affinity for protein kinase G. Accordingly, the suppression of renin release by these compounds is completely prevented by the pharmacological inhibition of the cGKs (254). Moreover, cGMP analogs inhibit renin secretion in the primary cultures of JG cells isolated from wild-type or cGKI-deficient kidneys, but not from cGKII-deficient kidneys (254, 927). Therefore, it can be concluded that cGMP can inhibit renin release via the activation of cGKI. The downstream targets of cGKII that eventually interfere with the exocytosis of renin storage granules are presently unknown.

The dual effects of cGMP on renin release raise the question of which factors determine whether endogenous cGMP stimulates or inhibits renin secretion. It is possible that the effects of cGMP depend on its intracellular concentration. Since low concentrations of cGMP preferentially inhibit PDE-3, the stimulation of renin release would occur. In contrast, high concentrations of cGMP can also activate cGKII. Since cGKII activation can, in principle, attenuate the stimulation of renin release in response to cAMP (480), a high concentration of cGMP would suppress renin release. Moreover, it appears likely that the stimulatory and inhibitory cGMP pathways are spatially segregated from each other. This hypothesis is consistent with the intracellular localization of the cGMP-generating guanylate cyclases and cGMP target enzymes. Soluble guanylate cyclase (sGC) is a cytosolic enzyme like PDE-3; as a result, the cGMP derived from sGC may predominantly stimulate renin secretion via the cAMP pathway. Since NO activates sGC, this pathway could provide an explanation for the stimulation of renin release by NO. In contrast to sGC, the membrane-bound particulate guan-
late cyclase (pGC) is found in close proximity to the vesicles associated with cGKII. Since cGMP derived from pGC would therefore inhibit renin secretion, this pathway could explain the inhibition of renin release in response to atrial natriuretic peptide (ANP) (see sect. vC4). Such a spatial segregation of cGMP signals induced by ANP and NO occurs in both HEK cells and vascular smooth muscle cells (623, 695), although no data supporting this hypothesis in JG cells are presently available.

IV. LOCAL CONTROL OF RENIN SECRETION

As will be outlined in section v, renin secretion is modulated by multiple systemic factors. In addition, the presence of renin-generating granular cells at the distal portion of the afferent arteriole enables the adjacent or nearby cells to exert a local control over the renin secretion. These local control mechanisms of renin secretion comprise tubular, vascular, and nervous components (Fig. 5). In addition to unambiguously locally generated factors, there are hormones such as ANP and ANG II that may be of local and/or systemic origin; those factors will be covered in section v.

A. Tubular Control of Renin Secretion

Renin secretion from the granular cells of the afferent arteriole is locally influenced by the tubular system of the corresponding nephron. The anatomical basis of this regulatory pathway for renin secretion resides in the close linkage of the tubular and vascular system within the confines of the JGA. Specifically, the thick ascending limb of Henle’s loop (TAL) emerging from the renal medulla comes into close contact with its parent glomerulus. This anatomical link between the tubular system and the vasculature of the afferent and efferent arterioles enables the specialized tubular cells of the cortical thick ascending limb of Henle’s loop (cTAL) and the macula densa (MD) cells located adjacent to the extraglomerular mesangium to influence renin secretion. In this local control system, changes in the tubular NaCl concentration are translated into inverse changes in renin secretion. Early experimental evidence for a tubular control mechanism of renin secretion has been reviewed elsewhere (293), but the most striking and direct evidence was provided by the following in vitro experiment. Rabbit JGAs were isolated, and TALs were perfused with artificial tubular fluid containing various concentrations of NaCl. Simultaneously, the entire JGA was superfused, and renin release was determined by microassay. In this experimental setup, a decrease in the tubular NaCl concentration resulted in “a prompt stimulation of the renin release rate” (815). This fundamental finding sparked interest in the signaling pathways that link the tubular NaCl concentration and renin secretion. The initial step of the MD-dependent control of renin secretion is the detection of the NaCl concentration in the tubular lumen by the MD cells. The transport activity of the Na\(^{+}\)-K\(^{+}\)-2Cl\(^{-}\) cotransporter NKCC2 (BSC1) in the apical membrane of these cells is thought to be the primary mechanism by which the MD cells detect the tubular NaCl concentration (64, 65, 500, 538, 640, 758, 958). In addition, the NaCl transport activity mediated by NHE2 might contribute to the MD salt sensing (306). The MD cells subsequently transmit information about the tubular NaCl concentration to the granular cells of the afferent arteriole to influence renin secretion. Three likely candidate molecules have been proposed to serve as mediators of the MD-dependent control of renin secretion: prostanoids, NO, and adenosine/ATP. These molecules meet the key criteria to serve as signal mediators between tubular epithelial cells and JG cells of the afferent arteriole: 1) MD cells have the capacity to gener-
Prostanoids are generated from arachidonic acid in a two-step reaction. The first step, the formation of prostaglandin H$_2$ (PGH$_2$), is catalyzed by two isoforms of cyclooxygenases, the constitutively expressed COX-1 and the inducible COX-2 (171, 660). PGH$_2$ is subsequently modified by the various prostanoid synthases to produce specific prostanoids. Of those, prostaglandin E$_2$ (PGE$_2$) and prostacyclin (PGI$_2$) were shown to directly stimulate renin secretion in isolated JG cells (241, 401).

A) Activation of EP$_2$, EP$_4$, and IP receptors stimulates renin secretion. In vitro investigations and studies in mice with a specific deletion of single prostanoid receptors revealed that the stimulation of renin secretion by PGE$_2$ and PGI$_2$ are mediated via EP$_2$, EP$_4$, and IP receptors (241, 244, 778). EP$_2$, EP$_4$, and IP prostanoid receptors are all coupled to G$_s$-mediated increases in intracellular cAMP, which act as the main stimulus of renin secretion (see sect. II C1).

B) Prostanoid generation in the TAL. Regarding the concept of local tubular control of the renin system, Harris et al. (315) first described the constitutive expression of COX-2 in MD cells of the rat. This finding suggested that COX-2-derived prostanoids might be involved in the MD control of renin secretion. An in-depth analysis of COX-2 expression in the cortical TAL by immunohistochemistry revealed that COX-2 expression in rats and rabbits is not restricted to the MD cells but is also found in the adjacent tubular cells of the TAL (151, 558, 866, 916). COX-2 expression in the TAL of primates and humans appears to be low compared with rodents, at least under normal conditions (429, 452, 621, 867). It should be noted in this context, however, that the typical diet in the Western hemisphere is rich in NaCl and that, as outlined later, COX-2 expression is suppressed by a high oral salt intake, which may thus impair the detection of COX-2 in human renal cortices by immunohistochemistry. In addition to COX-2, the MD cells also express the microsomal PGE$_2$ synthase, the downstream enzyme of PGE$_2$ synthesis (113, 454).

Whereas changes in prostanoid production due to the altered expression of COX-2 and accessory downstream enzymes are likely to influence renin secretion over longer time frames, an acute reduction of salt concentration in MD cells was shown to result in rapid changes in the prostanoid formation, presumably related to changes in COX-2 and PGE$_2$ synthase activity. In this context, in vitro experiments using the aforementioned biosensor approach indicated a release of PGE$_2$ across the basolateral membrane of the MD cells in response to a decreased tubular salt concentration or in response to the presence of loop diuretics (689). Such acute changes in the prostanoid formation require the presence of sufficient amounts of substrate, i.e., arachidonic acid. Whether these changes in phospholipase A$_2$ activity are causally involved in the modulation of the tubular prostanoid formation awaits experimental evaluation.

D) COX-2-derived prostanoids and renin secretion. Both in vitro and in vivo studies were performed to assess the functional relevance of prostanoid release from the MD cells in the control of renin secretion. In vitro experiments using isolated TAL/JGA preparations indicated a crucial role for MD prostanoid formation in the stimulation of renin secretion in response to a reduction in the salt concentration in the tubular perfusion fluid; that is, the nonselective blockade of COX activity blunted the increase in renin secretion following a reduction of the constitutive generation of prostanoids by MD cells would allow for a tonic influence on renin secretion by JG cells. However, the expression of COX-2 in the MD cells and the acute formation of prostanoids are regulated by physiological stimuli, making it feasible that the MD-derived prostanoids could be involved in the signal transmission between the epithelial MD and vascular JG cells. Most strikingly, the regulation of COX-2 is usually paralleled by corresponding changes in the activity of the renin system. Thus COX-2 mRNA expression in the MD is up-regulated upon chronic administration of loop diuretics (131, 414, 415). Similarly, Bartter patients (these patients suffer from loss-of-function mutations of NKCC2, or other crucial proteins for TAL NaCl reabsorption, such as CIC-NKB, ROMK, or Barttin) show markedly increased COX-2 expression in the cTAL, indicating a relationship between the NKCC2-dependent salt transport and COX-2 expression (453). COX-2 expression was also found to be increased under conditions of reduced oral salt intake or diminished renal perfusion pressure, both of which might result in a reduced distal TAL salt concentration (315, 318, 398, 955, 972). In addition, the enhanced expression of COX-2 observed in conditions of both reduced oral salt intake and compromised TAL salt transport activity (loop diuretics, Bartter’s patients) was paralleled by an increase in the expression of the microsomal PGE$_2$ synthase, the downstream enzyme of PGE$_2$ synthesis (113, 454).
tubular salt concentration (277). Similar results were obtained with the COX-2 inhibitor NS-398, whereas COX-1 inhibition had no effect (890). All attempts to assess the in vivo role of locally generated prostanoids in the regulation of renin secretion have been hampered by the lack of experimental tools to specifically or locally modulate the prostanoid formation within the confines of the JGA without inducing systemic effects. For example, the blockade of COX-2 activity during the inhibition of NKCC2 transport activity by the administration of loop diuretics markedly reduced the typical stimulation of renin secretion (414, 768). However, the reduced renin secretory response under loop diuretic treatment was accompanied by a considerably moderated diuretic effect of the loop diuretic, thus permitting no clear conclusion as to whether the blunted stimulation of renin secretion was directly or indirectly related to the COX inhibition. Similar results were found in humans with genetic inactivation of NKCC2: in type 1 Bartter’s patients, plasma renin activity and abnormal natriuresis/diuresis were both ameliorated by the specific inhibition of COX-2 with rofecoxib (723).

In healthy volunteers, COX-2 inhibition by rofecoxib was shown to block the increase in PRA in response to a salt-restricted diet (414). Initial animal experiments in rats and mice have suggested that the stimulation of renin secretion in response to various experimental maneuvers, including renal artery stenosis, ACE inhibition, AT1 receptor antagonism, and a salt-restricted diet, all depend on the intact COX-2-dependent prostanooid formation (311, 312, 934). In these early studies, experimental COX-2 inhibitors like NS398 and SC58236 were used. Follow-up studies using the Food and Drug Administration-approved COX-2 inhibitors rofecoxib or celecoxib were not able to fully confirm the initial results of reduced stimulation of renin secretion after oral salt restriction and after angiotensin antagonism (343, 346, 414). The somewhat contradictory results of the studies that sought to assess the in vivo role of prostanoids in the MD control of renin secretion might be attributed to a problem that appears to be inherent to all attempts to investigate the tubular control of renin secretion. More specifically, to date, we are not aware of any experimental protocol to specifically manipulate (which means reduce in the case of the stimulatory signaling pathway) the salt concentration at the MD segment of the TAL without causing systemic effects that, in turn, also affect the renin secretion.

The availability of COX-2-deficient mice provided a fresh impetus for the investigation of the tubular control of renin secretion. COX-2-deficient mice show markedly reduced baseline renin expression and secretion (152, 435, 969). In addition, the stimulation of renin secretion due to reduced oral salt intake, ACE inhibition, angiotensin AT1 receptor antagonism, and reduced renal perfusion pressure was reduced in COX-2 −/− mice compared with controls (152, 435, 969). Thus it was tempting to suggest that at least the MD-dependent component of the regulation of renin secretion under these conditions is highly dependent on the COX-2-dependent formation of prostanoids. However, the interpretation of the results was hampered by the fact that the COX-2 −/− mice suffer from retardation of renal development and considerable deterioration of renal function during adulthood (199, 600); additionally, the latter was shown to be highly dependent on the genetic background of the COX-2-deficient strain (971). Therefore, the regulation of renin secretion in COX-2 knockout mice was recently revisited in a study using COX-2-deficient mice of various genetic backgrounds (435). The baseline plasma renin concentration was reduced to some degree in all COX-2 −/− strains compared with the corresponding wild-type mice. Similarly, the renin secretory response to acute challenges like the administration of furosemide, ACE inhibitor, AT1 receptor antagonist, or isoproterenol was unanimously compromised in the COX-2 −/− mice compared with controls. However, when the baseline PRC of the COX-2-deficient mice was chronically augmented by pretreatment with a combination of an ACE inhibitor and a low-salt diet, the acute stimulation of renin secretion in response to furosemide was partially reestablished, suggesting that the COX-2-derived prostanoids are responsible in vivo for determining the size of the releasable pool of renin, which eventually defines the amount of renin that can be secreted during acute stimuli (435). Similar results were obtained in rats using the COX-2 inhibitor SC58236 (569), again indicating that COX-2-derived prostanoids act as general enhancers of the renin system, rather than as mediators of the regulatory input.

How can these results be reconciled with the credible in vitro data unequivocally showing that intact COX-2 activity is an indispensable prerequisite for the modulation of renin secretion in response to acute changes in the tubular salt concentration? Although this question cannot be answered comprehensively at the moment, accumulating evidence indicates that the role of the tubular control of renin secretion during chronic variations of the tubular salt concentration (e.g., chronic changes in oral salt intake) is limited, or it can be compensated for by other presumably systemic control mechanisms, which are absent in the situation of the in vitro JGA preparation; conversely, the MD seems to play a crucial role in the acute (inhibitory) control of renin secretion in vivo (435, 651, 652). We will further address this issue when we discuss the role of adenosine in the tubular control of renin secretion.

In summary, prostanoids like PGE2 and PGI2 are formed by tubular MD cells, and their generation is dependent on tubular salt concentration. PGE2 and PGI2 are potent stimulators of renin secretion and determine the operating point of the renin system in vivo. Their relevance as stimulatory mediators of a communication path-
way between the TAL and JG cells in vitro (i.e., in the absence of other regulatory mechanisms) is well established, although their significance as regulatory factors of renin secretion in vivo is less clear.

2. NO

NO activates both inhibitory and stimulatory pathways of renin secretion in the granular JG cells, as outlined in detail in section III. However, with all of the relevant data taken together, it now appears well established that the overall effect of NO on renin secretion, at least as far as the in vivo situation is concerned, is stimulatory (407, 683, 765, 770).

A) NOS IN THE VICINITY OF RENIN-GENERATING CELLS. The formation of NO from L-arginine is catalyzed by three different NOS isoforms (936, 937). At least two of the three known NOS isoforms are expressed in close vicinity to the granular cells of the afferent arteriole. Endothelial NOS (eNOS) is localized to the endothelial cells of the afferent arteriole, and nNOS is found in the MD of the TAL (608, 952). Commensurate with a possible role of NO as a mediator of the MD-dependent control of renin secretion, the expression of nNOS in the MD was shown to be regulated in parallel with the expression of renin under various conditions, including the variation of oral salt intake, administration of loop diuretics, and renal hypoperfusion (84, 126, 611, 772, 808, 886).

B) MACULA Densa NO FORMATION AND RENIN SECRETION. A role for NO as a mediator of the MD-dependent stimulation of renin secretion was first suggested in a study that utilized isolated perfused JGA preparations (326). The increase in renin secretion observed in response to a low-salt concentration in the artificial perfusate of the TAL was markedly attenuated in the presence of the nonspecific NOS inhibitor Nω-nitro-L-arginine (326). For in vivo studies with the specific nNOS inhibitor 7-nitroindazole (7-NI), the results were less clear. More specifically, some studies reported that 7-NI was capable of eliminating or at least reducing the effect of a low-salt diet on the renin system (60, 854), whereas another study found no influence of 7-NI on the renal renin content given a salt-restricted diet (312). Several studies have assessed the effect of NOS inhibition on the stimulation of renin secretion caused by loop diuretics, which were used as a measure to address the MD-dependent control of renin secretion (325). In an isolated renal vessel preparation, NOS inhibition substantially reduced the increase in renin release after pretreatment with furosemide (142). Similar results were obtained in vivo, where renin secretion was reduced in response to loop diuretics under concomitant NOS inhibition (61, 722, 768). The interpretation of the results derived from studies with a pharmacological inhibition of NOS is complicated because it is unclear to what extent the agents specifically inhibit each of the three NOS isoforms. In particular, the specificity of the widely used agent 7-NI for nNOS over eNOS and inducible NOS (iNOS) is based on its preferential in vivo uptake by neuronal cells compared with endothelial cells, rendering its specificity for nNOS in cells other than neurons questionable (597). To circumvent these issues and to assess whether MD-derived NO is a functional stimulatory mediator of renin release in response to changes in the tubular salt content, the effect of a loop diuretic on renin secretion was investigated in nNOS- and eNOS-deficient mice (130). The loss of either nNOS or eNOS activity had little impact on the magnitude of renin secretion in response to furosemide application. However, after the nonspecific inhibition of all NOS isoforms by Nω-nitro-L-arginine methyl ester (L-NAME), the furosemide-induced renin secretion was substantially reduced in wild-type, nNOS −/−, and eNOS −/− mice. These data suggest that NO acts as an enhancer of renin secretion irrespective of its source, and therefore, NO is not required to mediate the MD control of renin secretion. Similarly, in the isolated perfused kidney, the effect of bumetanide on renin secretion was blunted by NOS inhibition. However, clamping the NO concentrations in the isolated perfused kidney by exogenous NO administration with the concomitant inhibition of all NOS isoforms fully restored the renin secretory responsiveness to bumetanide, suggesting again that the mere presence of NO is sufficient and that a fluctuation in NO concentrations is not required for the activation of the MD control of renin secretion (130).

In summary, NO is generated in the vicinity of the JG granular cells by nNOS and eNOS and serves as a stimulatory, permissive factor in renin secretion. On the basis of the present data, it seems unlikely that NO derived from MD cells functions as a mediator of the tubular control of renin secretion.

3. Adenosine/ATP

Adenosine meets the key requirements to serve as a mediator of the local tubular control of the renin system. First, there is solid evidence that adenosine is generated in the JGA. Second, the formation of adenosine in the JGA depends on the tubular salt concentration in the MD segment of the TAL. Third, adenosine exerts a direct effect on renin secretion from JG granular cells of the afferent arteriole.

A) TUBULAR NaCl CONCENTRATION AND ADENOSINE FORMATION IN THE JGA. The function of adenosine as a paracrine factor in the JGA has been the subject of intense research over the past several years. The focus, however, has been on the role of adenosine in the MD control of pregglomerular resistance (the so-called tubuloglomerular feedback mechanism; Ref. 874) rather than its function in the local tubular control of renin secretion. Nevertheless, progress
made towards the elucidation of the signaling pathways that connect the tubular MD cells with the vascular smooth muscle cells of the afferent arteriole also expanded our knowledge of the local tubular control of renin secretion. The data indicate that the following chain of events is initiated by an increase in the tubular salt concentration at the macula densa. First, MD cells detect increases in the tubular NaCl concentration via enhanced NKCC2-dependent NaCl reabsorption, the MD cells subsequently release ATP via their basolateral membrane, and the ATP is successively dephosphorylated in the extracellular space to eventually form adenosine, which in turn causes afferent arteriolar vasoconstriction and a concomitant drop in the GFR of the respective nephron (66, 90, 125, 455, 650, 832). A1 adenosine receptors (A1AR) are expressed in vascular smooth muscle cells of the afferent arteriole, with particularly high expression levels in the most distal portion of the vessel (390), and they mediate the vasoconstriction through the G_{i}dependent activation of phospholipase C (308). Since the granular JG cells also express A1AR (11), it is conceivable that adenosine may also influence or suppress renin secretion in response to increases in the tubular NaCl concentration. In fact, the dose-dependent inhibition of renin secretion by adenosine was demonstrated for isolated JG cells and renal cortical slices (160, 474). The A1AR antagonists such as 8-cyclopentyl-1,3 dipropylxanthine (DPCPX) in turn result in a stimulation of renin secretion, suggesting that the renin system is tonically suppressed by endogenous adenosine formation (388, 469, 470). The assumptions that renin secretion is controlled by an endogenous “adenosine-brake” (388) and that adenosine in this context is derived from tubular sources, i.e., tubular ATP, are further supported by the observation that renin secretion in the isolated JGA is lower in the presence of the tubular MD segment than in its absence. This difference in the renin release rate was eliminated by the nonspecific adenosine receptor antagonist theophylline (381). Similarly, the PRA was increased in humans after the application of the A1AR antagonist FK-453, again suggesting a tonic inhibition of renin release by adenosine (43). The involvement of adenosine in the mediation of the MD-dependent suppression of renin secretion in response to high tubular NaCl was first suggested by in vitro experiments using the isolated TAL/JGA preparation. In this experimental setup, as mentioned above, an increase in the salt concentration of the tubular perfusion fluid resulted in a reduction of the renin release rate (815). The application of DPCPX to the bath markedly reduced the magnitude of this decrease in renin secretion, indicating that the activation of A1AR by adenosine constitutes a crucial step in the signaling cascade between tubular cells and the effector cells of the afferent arteriole (943).

The in vivo significance of adenosine in the tubular control of renin secretion was assessed in studies using A1AR-deficient mice (90, 832). The effects of acute changes in the tubular salt concentration were investigated in these mice by acute salt loading to raise the NaCl concentration at the MD or by the application of loop diuretics to mimic a low tubular salt concentration. The acute salt loading was achieved by the intravenous application of isotonic saline, an experimental maneuver that has been shown to result in increased distal tubular salt concentration and a concomitant suppression of renin secretion (440, 537). In A1AR−/− mice, the acute suppression of renin secretion provoked by acute salt loading was completely absent, whereas the PRC was reduced in wild-type controls by ~40% (436). The converse of salt loading, a blockade of the MD salt transport by the use of furosemide, resulted in a comparable stimulation of renin secretion in both wild-type and A1AR-deficient mice, suggesting that the adenosine-dependent signaling is responsible for the inhibitory arm of the tubular control of the renin system (436). In contrast to acute changes in the tubular salt, the chronic modulation of oral salt intake does not appear to be dependent on intact adenosine signaling. Thus the stimulation and suppression of the renin system under dietary salt-deprived conditions were fully preserved in the A1AR−/− mice (784).

The assumption that this adenosine-dependent suppression of renin secretion derives from the tubular control of the renin system is further supported by the observation that the acute suppression of renin secretion due to salt loading is also absent in NKCC2A-deficient mice, a strain that lacks the isoform of NKCC2 responsible for MD salt-sensing in the high concentration range (651, 652). However, both these and the A1AR-deficient mice display a reduction in the PRC after chronic high salt intake, similar to that observed in wild-type controls. Thus A1AR- and NKCC2A-deficient mice maintain an inverse regulation of PRC after a high-salt diet, but they have lost the ability to suppress PRC after acute salt loading (652). Since NKCC2 expression is restricted to the TAL (211, 640, 970), these data again suggest that the negative tubular control of renin secretion is more relevant for acute changes in tubular NaCl than for chronic changes and that a lack of tubular control can be compensated for by other factors when salt intake is chronically increased.

B) ADENOSINE AND PRESSURE-DEPENDENT REGULATION OF RENIN SECRETION. With respect to the pressure-dependent control of renin secretion, the contributions of local tubular, vascular, and systemic factors remain to be determined (see also sect. iv.B). The experimental evidence suggests a crucial role for the adenosine-dependent signaling in the tubular domain. Thus the acute and chronic phenylephrine infusion in vivo resulted in a suppression of PRC in wild-type mice, but not in A1AR-deficient mice, despite similar increases in the arterial pressure (783). Similarly, in the isolated perfused kidney, the A1AR deficiency and
inhibition of $A_\beta$AR by DPCPX eliminated the suppression of renin secretion in response to a stepwise increase in the renal perfusion pressure (783). Conversely, renin secretion was enhanced by a decrease in the perfusion pressure, irrespective of whether functional $A_\beta$AR was present or not (783). The results of this in vitro study resemble an in vivo experiment in which the vasodilator agent hydralazine was used to reduce the arterial pressure. Again, PRC was enhanced to a similar extent after hydralazine administration in $A_\beta$AR $\sim$/$\sim$ mice and wild-type controls (436). These data suggest that intact adenosine signaling is critical to the pressure-dependent inhibitory arm of renin secretion, whereas other factors (rather than decreased adenosine formation) appear to be responsible for the stimulatory arm, which is activated by low arterial pressure.

B. Vascular Control of Renin Secretion

JG granular cells in the afferent arteriole are surrounded and influenced by neighboring vascular smooth muscle cells and endothelial cells (see also sect. III). Multiple local factors derived from the vasculature may influence renin expression and secretion, as summarized in detail elsewhere (293). We focus on four major local factors that appear to be primarily involved in the communication among vascular smooth muscle cells, endothelial cells, and JG cells: NO, adenosine/ATP, endothelins, and prostanoids. In this section, we also assess the data that relate to the regulation of renin secretion by the local “baroreceptor,” although it is still difficult to define whether and to what degree the local baroreceptor is related to vascular or tubular events or events that occur within JG cells.

1. NO

Due to the expression of eNOS, the endothelium is a rich source of NO in most vascular beds, including the kidney. Specifically, the expression of eNOS in the afferent arteriole has been shown for a number of mammalian species (40, 41, 896). A coculture of JG cells with endothelial cells revealed both inhibitory and stimulatory signaling derived from the endothelial cells, with the stimulatory component related to endothelium-dependent NO formation (462, 481, 773). In contrast, the NOS inhibition in the absence of endothelial cells had no effect on the renin secretion from JG cells, presumably due to the lack of NOS expression in JG cells (773). The relevance of endothelium-derived NO for the release of renin was further supported by studies in the isolated perfused kidney. The addition of acetylcholine, a known stimulator of endothelial NO release (248), to the perfusion fluid increased the renin release rate, which was blunted by a concomitant NOS inhibition (610, 765). Furthermore, eNOS was shown to have a considerable tonic stimulatory influence on renin transcription in vivo without being involved in the regulation of renin mRNA expression under conditions such as a high-salt or low-salt diet in combination with the ACE inhibitor ramipril (923). Finally, as mentioned above, in vivo data from eNOS- and nNOS-deficient mice suggest that the adequate NO generation in the vicinity of JG cells, irrespective of whether the source is endothelial (eNOS) or tubular (nNOS), constitutes a permissive factor that allows other regulatory mechanisms of PRC to operate at full magnitude (130).

2. Adenosine/ATP

The function of adenosine in the local control of renin secretion was touched upon in connection with the tubular mechanisms that account for the adenosine-dependent suppression of renin secretion. The formation of adenosine in the confines of the JGA, however, is not limited to the ATP release by MD cells and the subsequent extracellular dephosphorylation resulting in the formation of adenosine (66, 455). ATP is also known to be released by endothelial cells and vascular smooth muscle cells, e.g., in response to increased shear stress (80, 582, 678, 786, 938). In fact, the renal extracellular ATP concentration as determined by microdialysis has been shown to be directly related to the renal perfusion pressure, which was most likely related to both tubular and vascular ATP release (631, 632). Of note, some regulatory capacity of renin secretion by the renal perfusion pressure was preserved in hydronephrotic kidneys, i.e., in the absence of functional tubular-JG signaling (766). Thus the vasculature-dependent ATP release plays a key role in mediating the renal autoregulation in response to increased perfusion pressure (375, 376, 554). In addition, JG cells were shown to release ATP in response to mechanical stretching (973); in this context, ATP could subsequently either influence JG cells in an autocrine manner, or ATP might be subject to dephosphorylation in the extracellular space. In terms of an increased renal perfusion pressure, the overall effect of ATP released by the endothelial, smooth muscle, or JG cells is supposedly the suppression of renin secretion (973). Since ATP, however, has been shown to stimulate renin secretion from renal cortical slices, presumably via enhancing endothelial NO generation (162), it is likely that the bulk of the ATP present in vivo is locally hydrolyzed to form adenosine, which then acts on $A_\alpha$AR (160, 474), or that the ATP acts directly on the P$_2$ purinoreceptors present on JG cells (973).

In summary, the vascular release of ATP and subsequent formation of adenosine in the extracellular space appears to contribute to the local adenosine formation, which results in a suppression of renin secretion mediated by the activation of $A_\alpha$AR. This may be of particular interest...
relevance during situations of increased renal perfusion pressure.

3. Adenosine/ATP and renal baroreceptor

The term renal baroreceptor describes the inverse relationship between the intrarenal (perfusion) pressure and renin secretion. Although it is still difficult to pinpoint the exact nature of the renal baroreceptor, it appears likely that the adenosine/ATP from vascular and tubular sources is crucially involved in the suppression of renin secretion during increased intrarenal pressure, as mentioned above. In addition, mechanical stretching results in the release of ATP from JG cells (973). This release of ATP from JG cells may provide a tubule-independent link between renal perfusion pressure and renin secretion and may participate in the still elusive local “baroreceptor.” Multiple studies describe a stretch or transmural pressure dependency of JG function. Thus in isolated JG cells, the mechanical stretch reduces both the basal and prestimulated renin secretion in parallel with reduced renin transcription (117). In addition, the processing of pro-renin into active renin is attenuated by transmural pressure on JG cells (339). Similar results were obtained for the renin-expressing cell line As4.1 after mechanical elongation (736). In this study, the activity of a renin promoter reporter construct was diminished upon mechanical stretching. The mechanical stretching of the As4.1 cells also induced a rise in the intracellular calcium, the well-established intracellular inhibitor of renin formation (see sect. IVD) (738). Contrary to this report, however, the mechanical stretch of mouse JG cells in situ in isolated afferent arterioles did not affect the JG cell calcium levels (761).

In summary, the investigation into the mechanisms underlying the local renal baroreceptor and the pressure-dependent control of the renin system requires further experimental efforts. It appears most likely that the baroreceptor is composed of several factors, including tubular, vascular, and JG cell-related components.

4. Endothelins

Multiple studies have suggested that endothelins serve as negative regulators of renin expression and secretion. A primary culture of JG cells revealed that endothelin-1, -2, and -3 all inhibit the increase in renin secretion that occurs after the stimulation of the cAMP pathway, whereas they have little influence on the baseline renin secretion. The in vitro effect of endothelins on the cAMP-stimulated renin secretion was not modulated by the ET<sub>A</sub> receptor antagonist BQ123. Conversely, the ET<sub>B</sub> agonists like IRL-1620 and sarafotoxin S6b had similar effects on the renin secretion as those elicited by endothelins, suggesting that the endothelins influence renin secretion via activation of the ET<sub>B</sub> receptors (4, 728). Similar results were obtained with regard to renin gene expression (727). For the isolated perfused kidney, a slight increase in the renin release rate was observed at a low endothelin concentration (10 pM), whereas higher concentrations consistently suppressed renin secretion; once again, this effect was primarily mediated by ET<sub>B</sub> receptors (763). It is conceivable that the activation of the ET<sub>B</sub> receptors exerts a dual effect on renin secretion, with a stimulatory component related to the enhanced endothelial NO formation (218, 337) and a direct inhibitory component that depends on the ET<sub>B</sub> receptors present on JG cells. The correlation between endothelins and the renin system in vivo was assessed during chronic hypoxia in rats (782). The ET receptor antagonist LU135252 administered during chronic hypoxia resulted in a marked increase in renin gene expression, suggesting a negative effect of endothelins on the renin system and basically confirming the in vitro data (782).

At present, our knowledge of the functional impact and relevance of the endothelin system on the regulation of renin expression and secretion is limited. Endothelins apparently act as endothelial factors that tonically counteract the stimulatory effects of other endothelium-derived factors, most notably NO. Since the endothelial generation of endothelin-1, in particular, was shown to be related to vascular shear stress (733), it appears reasonable to suggest an involvement of the endothelin system in the pressure-dependent regulation of renin secretion, a possibility that awaits further experimental evaluation.

5. Prostanoids

The role of prostanoids in the control of renin expression and secretion was discussed in detail in the context of tubular prostanoid formation. However, in addition to the tubules, the renal vasculature also appears to be a significant source of prostanoids, particularly prostacyclin. In humans, the substantial expression of COX-2 was observed in the afferent arteriole in close vicinity to the granular JG cells, which exceeded the expression of COX-2 in the TAL (867). In rodents, conversely, no COX-2 but some COX-1 was found in the renal vasculature (113). The vascular expression of COX-2 in humans was enhanced in patients with renal artery stenosis, and therefore, vascular COX-2 activity may contribute to the increased renin generation during renal hypoperfusion. Similarly, in mice, the formation of prostacyclin and the activation of the IP receptors appear to be crucial for the activation of renin secretion during renal hypoperfusion. Accordingly, the stimulation of the renin system during renal artery stenosis was markedly reduced in IP receptor-deficient mice (244). In addition, stimulation of the renin system after the application of loop diuretics may be at least partly related to the enhanced vascular prostacyclin formation (518). Conversely, the attenuation of renin secretion in
response to loop diuretics by inhibitors of cyclooxygenases appears to be partially mediated by the reduced vascular prostacyclin synthesis (344, 414, 415, 827).

In conclusion, prostanooids derived from vascular sources in the vicinity of JG cells (or from the systemic vasculature, as outlined in section V) constitute potent stimulators of renin secretion and expression. Their contribution to overall prostanooid formation may be considerably species dependent. Changes in the formation of prostanooids, e.g., in response to decreased renal perfusion pressure, are not restricted to changes in the tubular prostanooid formation, but they are often paralleled by regulated vascular prostanooid formation.

C. Neural Control of Renin Secretion

The presence of β₁-adrenergic receptors in the JG granular cells has been established by multiple receptor binding studies and by in situ hybridization. The results of these investigations are summarized in detail elsewhere (293). More recently, the expression of β₁-receptors on JG cells was shown by immunohistochemistry (81). The activation of β₁-receptors by catecholamines results in an augmented formation of cAMP, the central intracellular stimulus of renin secretion (see sect. III). Thus the pharmacological inhibition of β₁-receptors results in reduced renin secretion (130, 423). Similarly, renal denervation to excise sympathetic input was shown to lower renin expression (271, 354), but it should be noted that renal denervation also removes the input of the sympathetic system mediated by α-receptors, including hemodynamic and tubular effects (335). Most notably, double-knockout mice deficient in β₁- and β₂-receptors have substantially reduced basal PRC, ~85% lower than that observed in wild-type controls (434). Thus the sympathetic input from local nerve endings or from circulating catecholamines may represent the most powerful background stimulus for renin secretion. In view of the significance of the sympathetic nerve tone for the maintenance of basal renin secretion, it appears reasonable to also suggest an involvement of sympathetic input in the regulation of renin secretion triggered by changes in oral salt intake. It should be mentioned in this context that the renal sympathetic nerve activity is higher in rats on a low-salt diet than in those on a high-salt diet (118). However, most studies suggest that modulation of the activity of the renin system is not crucially dependent on intact β-adrenergic input. That is, most reports indicate that neither renal denervation nor pharmacological β-blockade considerably influence the stimulation of the renin system by a salt-deficient diet or its suppression by a high-salt diet (271, 353, 354). In some studies in humans, dogs, and rats, however, the β-receptor antagonist propranolol was shown to reduce the magnitude of the renin system stimulation during a salt-deprived diet (423, 879). These somewhat contradictory results might be attributed to differences in the species or in the specific experimental setups used. However, for most in vivo studies utilizing β-antagonists, it has remained unclear to what extent β-inhibition was achieved. Those issues were circumvented by the use of β₁/β₂ double-knockout mice (434). As mentioned above, basal renin expression and PRC in β₁/β₂ −/− mice were both substantially reduced compared with wild-type controls. In β₁/β₂ −/− mice, the changes in renin secretion that occurred in response to both acute and chronic stimuli, such as oral salt intake, loop diuretics, ACE blockers, and AT receptor antagonists, were maintained; however, the magnitudes of these changes in the PRC were markedly diminished (434).

In summary, activation of JG cell β₁ receptors is required to sustain basal renin expression and secretion. Furthermore, the β₁ receptors are necessary to drive the renin synthesis needed to maintain a releasable renin pool, which provides the basis for the full-magnitude secretory responses to external stimuli.

V. SYSTEMIC CONTROL OF RENIN SYNTHESIS AND SECRETION

The RAAS is critically involved in the regulation of salt and volume homeostasis and in the control of blood pressure. These variables in turn influence renin synthesis and renin release via negative-feedback loops. In addition, renin synthesis and release are also influenced by sympathetic nerves, tubular mechanisms, renal autacoids, and hormones. We discuss, in this section, the newest advances relevant to the regulation of renin release and synthesis.

A. Blood Pressure

The regulation of blood pressure involves a variety of organ systems including the central nervous system (CNS), cardiovascular system, kidney, and adrenal glands. These systems modulate cardiac output, fluid volume, and peripheral vascular resistance as the major determinants of blood pressure. The renin-angiotensin system is a key regulator of blood pressure and salt and water homeostasis (946). The inhibitors of ACE or ANG II receptor antagonists are potent blood pressure-lowering drugs that may also attenuate cardiovascular and renal injuries (945, 954). Due to changes in the formation of the vasoconstrictor ANG II, alterations in renin release can also cause changes in the arterial blood pressure, such that an increased rate of renin release increases blood pressure and vice versa. Accordingly, renin inhibitors have been shown to lower blood pressure to similar levels as ANG II-AT₁ receptor blockers or ACE inhibitors (544). Furthermore, mice with genetic deletions of angiotensinogen, renin, ACE, and
AT$_{1a}$ receptors (in contrast to humans and all other mammalian species, rodents possess two AT$_1$-receptor isoforms: AT$_{1a}$ and AT$_{1b}$) are hypotensive (220, 380, 432, 466, 967). Virtually all tissues presumed to have a major role in blood pressure control, including the heart, the vasculature, the adrenal glands, the nervous system, and the kidney, express AT$_1$ receptors (164). Via activation of the AT$_1$ receptors, ANG II triggers multiple effects, such as vasoconstriction, stimulation of aldosterone release, central vasopressor responses, and increased renal sodium retention (8, 30, 182, 230, 877), which may potentially lead to an increase in the systemic blood pressure. It has been assumed that the body has several important systems for controlling blood pressure, which react within seconds (baroreceptors, chemoreceptors, and CNS ischemic response), minutes (the RAAS, stress relaxation, capillary fluid shift, and aldosterone release), and finally hours or days (renal volume control) (291). Nevertheless, it has been suggested that the kidney is the dominant mechanism for the long-term regulation of blood pressure; as such, an elevation in the renal perfusion pressure due to an increased arterial blood pressure enhances sodium and water excretion, leading to a decrease in body fluid volume and vice versa (“pressure natriuresis”) (291). Moreover, renal transplantation studies further support the central role of the kidney in regulating blood pressure (725). The RAAS modulates renal function such that the activation of the RAAS causes a shift of the pressure-natriuresis curve to the right and the inhibition of the RAAS causes a shift to the left. This was demonstrated, for example, by showing that the infusion of ANG II into the renal artery reduced the sodium reabsorption and increased blood pressure without impairing peripheral resistance (300, 302). A critical role for renal AT$_{1a}$ receptors derives from the AT$_{1a}$ receptor-deficient mice, which develop a salt-sensitive hypertension (646). Recently, renal cross-transplantation studies with AT$_{1a}$ receptor-deficient mice and wild-type mice further demonstrated the importance of renal AT$_{1a}$ receptors for blood pressure control. The specific absence of renal AT$_{1a}$ receptors (“renal knockout”) lowered blood pressure from ~118 mmHg in wild-type mice to 99 mmHg in renal knockouts, suggesting that the kidney is an important determinant of blood pressure control (173). However, there is also evidence of a role for the RAS in the control of blood pressure in nonrenal tissues. In this context, it has been found, for example, that the injection of ANG II into the brain increases blood pressure, likely via activation of the AT$_{1a}$ receptors (15, 182), and that the systemic infusion of ANG II induces vasoconstriction via the activation of vascular AT$_{1a}$ receptors. To address the role of extrarenal tissues in the control of blood pressure, Crowley et al. (173) also investigated a “systemic AT$_{1a}$-receptor knockout” with intact renal AT$_{1a}$ receptors; they found that blood pressure decreased to the same extent as for renal knockouts. Because blood pressure was further reduced to ~86 mmHg in animals that completely lacked the AT$_{1a}$ receptors, these findings suggest that both renal and extrarenal AT$_{1a}$ receptors contribute to blood pressure control (173). Subsequently, the authors showed that ANG II-induced hypertension depends on the presence of intact renal AT$_{1a}$ (172).

As part of a negative-feedback loop, blood pressure in turn affects the synthesis and release of renin from the JG cells of the kidney: an increase in the arterial blood pressure inhibits, whereas a decrease stimulates, the synthesis and secretion of renin (925). This pressure-dependent mechanism, which controls renin synthesis and release, was denoted the “long negative-feedback loop” (293). Increases in the systemic blood pressure can inhibit renin release via intrarenal as well as extrarenal mechanisms. Both mechanisms have been suggested to be involved in the pressure-dependent regulation of the renin system. With regard to intrarenal mechanisms, high blood pressure 1) induces pressure-dependent natriuresis, which increases the NaCl load at the macula densa, and 2) increases the renal perfusion pressure and therefore influences the renal baroreceptor mechanism.

Since the effects of blood pressure on renin secretion also occur in the isolated kidney (765), it seems likely that the renal baroreceptor mechanism could be involved in the pressure-dependent regulation of renin release. Although the precise identity and localization of the renal baroreceptor are still unclear, this sensing “receptor” may be located within the afferent arteriole. Much effort has been spent in attempting to discriminate between the pressure-dependent effects and the MD mechanism. However, the baroreceptor response was shown to be independent of the tubular (MD-mediated) effects (293), although the MD may still participate in the pressure-dependent effects (766, 767). In addition, it has been shown that the renal perfusion pressure, not the renal perfusion flow, is important for renin secretion (617). As outlined before, several renal autacoids have been shown to modulate the pressure-dependent regulation of the renin system, with roles for NO and prostaglandins in response to a drop in the renal perfusion pressure and for adenosine in response to an increased arterial blood pressure (see sect. nB for details). The renal cross-transplantation studies with AT$_{1a}$ receptor-deficient mice may also provide further insight into the regulation of renin by the renal baroreceptor mechanism. The renal AT$_{1a}$-receptor knockouts had normal renin mRNA levels, despite the absence of AT$_{1a}$ receptors at the JG cells and a significantly lower blood pressure, two factors that have been suggested to increase renin synthesis and renin release. However, renin strongly increased in the complete knockouts, which was paralleled by a further decrease in blood pressure (173). Therefore, one may assume that the renal baroreceptor mechanism may be primarily responsible for the
strong rise in renin mRNA in the total AT₁ receptor knockouts. Because blood pressure reductions of ~20 mmHg did not increase renin mRNA, these data further suggest that a threshold of blood pressure lowering must be achieved to stimulate the renin system via the renal baroreceptor mechanism, as was anticipated in earlier studies (439).

An important extrarenal mechanism involved in the control of renin secretion is the renal sympathetic nerve activity (RSNA). The autonomic nervous system, via the renal sympathetic nerves, adjusts kidney function dynamically in response to changes in the sensory information arising from the cardiovascular system, the soma, viscera, and the higher cortical centers. The renal sympathetic nerves innervate the vascular and tubular components, thereby regulating the renal hemodynamics, salt and water reabsorption, and renin secretion (868). The kidney has dense sympathetic innervations of vascular smooth muscle cells of the afferent and efferent arterioles, including the JG cells, as well as of most of the tubular segments in the cortex, including the proximal tubule, the loop of Henle, and the distal tubule (46, 194). Increases in the RSNA increase renin secretion, decrease urinary sodium excretion, and decrease the renal blood flow and GFR (194, 511, 557). With the use of graded frequency renal sympathetic nerve stimulation, it was found that the frequency response curve for renin secretion was to the left of that for decreases in sodium excretion, which, in turn, was to the left of that for decreases in renal blood flow. Therefore, it was assumed that an increase in renin secretion could be achieved before antinatriuresis and renal vasoconstriction occurs (194, 406). However, a resulting increase in ANG II due to the increased release of renin by the low levels of renal nerve activation could itself affect urinary excretion. In a recent study, it was shown that a low-level stimulation of renal nerves resulted in an ANG II-dependent increase in blood pressure and a decrease in the renal blood flow, urine flow rate, and sodium excretion, which was attenuated by ACE inhibition. Therefore, these data suggest that the changes in urinary excretion in response to activation of the renal nerves are mediated directly by the renal nerves and indirectly by an increase in ANG II, which may be due to an increase in renin secretion (502).

Afferent inputs to the CNS from the somatosensory receptors, cardiovascular baroreceptors, visceral receptors, renorenal reflexes, and higher cortical centers modulate the RSNA. Cardiovascular baroreceptors have been suggested to be of major importance for afferent inputs. The deactivation of high pressure receptors of the carotid sinus and the aortic arch led to an increase in the RSNA in response to a fall in blood pressure (119), and the low pressure receptors of the cardiopulmonary area inhibit the RSNA in response to an increase in volume within the vascular system (196). It is well known that stimulation of the left atrial receptors leads to a reflex reduction in the RSNA and the inhibition of renin release (298), and a fall in the systemic blood pressure unloads the carotid baroreceptors and leads to the stimulation of renin release (195, 868). However, the increased renin secretion in response to hypotensive hypovolemia does not seem to depend on the cardiac or arterial baroreceptors (704, 872, 873, 932).

The arterial baroreceptors have long been considered to be only crucial for the short-term control of blood pressure (169, 869). However, in a new model of chronic baroreceptor unloading, it has been found that blood pressure, heart rate, and plasma renin activity increase in response to baroreceptor unloading, suggesting that arterial baroreceptors are also involved in the long-term control of blood pressure (870, 871). Additional supporting evidence derives from the studies of Lohmeier et al. (527), who investigated the responses to five days of ANG II infusion in dogs using a split-bladder preparation combined with the denervation of one kidney. During ANG II infusion, the sodium excretion from the innervated kidney increased compared with the denervated kidney, indicating a chronic decrease in the RSNA in response to ANG II. This effect seemed to be mediated by the baroreflexes. In a subsequent study, they found an increase in sodium excretion in the innervated kidney in response to chronic ANG II infusion (528). Similarly, it has been found that the decrease in RSNA in response to chronic infusion of ANG II in rabbits is baroreceptor mediated (48, 49). Thus these data further support the role of arterial baroreceptors in the long-term regulation of blood pressure. Moreover, these results suggest that the baroreflexes may actually inhibit the renal sympathetic nerve during ANG II-induced hypertension, and in the absence of these reflexes, ANG II has sustained renal sympathoexcitatory effects. Accordingly, it has been found that the chronic activation of the carotid baroreflex reduces blood pressure, heart rate, and plasma norepinephrine levels. However, the plasma renin activity was unchanged, despite a strong fall in blood pressure, suggesting the presence of an inhibitory influence, likely baroreflex-mediated renal sympathoinhibition, on the renin release during sustained activation of the carotid baroreflex (526). This assumption is supported by acute observations in chronically instrumented dogs (686). Surprisingly, bilateral renal denervation had no impact on the blood pressure response and on renin secretion following prolonged baroreflex activation (524). Therefore, the presence of renal nerves does not seem to be an obligate requirement for long-term reductions in the arterial pressure and renin release during prolonged activation of the baroreflex.

Furthermore, the renal nerve activity, as well as arterial baroreflex control, can be modulated by ANG II within the CNS (193, 338, 566, 741). In addition, several other sensory receptors, including nociceptors, metabo-, mechano-, osmo-, chemo-, and thermoreceptors, can in-
fluence the level of sympathetic nerve activity and, consequently, the renin release and synthesis (195, 461).

In summary, blood pressure is an important regulator of renin release and synthesis. The blood pressure-dependent renin release is influenced by the activity of the renal nerves, by baroreceptor reflex loops and renal perfusion pressure, which in turn control the renin release via the intrarenal baroreceptor mechanism.

B. Salt Intake

Oral salt intake is a well-known important regulator of RAAS activity, such that low salt intake increases and high salt intake decreases renin synthesis and secretion (925). Recently, it was found that sodium intake determines the activity of the renin system in a log-linear fashion (441). The salt-dependent changes in the RAAS activity have been suggested to be mediated by 1) systemic humoral factors, 2) intrarenal factors, 3) renal nerves, or 4) the intrarenal MD mechanism. At present, however, it is still unclear which factor(s) or mechanism(s) mediates the observed salt-induced changes.

The role of local factors, including the intrarenal MD mechanism, locally acting mediators, and renal nerves, in the salt-induced changes in the renin system was discussed above in detail. As outlined there, the MD-dependent (tubular) control of renin secretion in response to changes in the luminal NaCl concentration seems to be more relevant for acute than for chronic changes in the tubular NaCl. Furthermore, locally generated mediators like the COX-2-derived prostanoids and NO act as modulators of the RAAS activity rather than as major mediators of the long-term salt-dependent control of the renin system (see sect. IV A). Several studies have indicated a suppression of the sympathetic nervous system with high salt intake (25, 196, 525). Therefore, the inhibition of the RAS in response to high salt intake may be due to a reduction of the RSNA. More recently, however, it has been found that a high-salt diet decreased the PRA independent of changes in blood pressure, was followed by natriuresis without changes in blood pressure, and in one study, this effect even occurred together with a fall in blood pressure (22). These findings suggest that the response to sodium load may take place in the absence of increases in blood pressure, and therefore, the pressure-natriuresis concept may be inadequate (75). In addition, it was shown that the natriuresis in response to sodium load, which increased blood pressure, was inhibited by the administration of ANG II, suggesting that a decrease in the RAS is a prerequisite for the natriuretic response to sodium load (21, 74). Subsequent elegant studies in humans and laboratory animals further suggest that the suppression of renin release in response to an acute modest sodium load is not only independent of changes in blood pressure, but also independent of cardiac output, GFR, ANP, β1-adrenergic receptors, plasma sodium levels, osmolality, renal nerves, and nNOS-derived NO (73, 456, 593, 717). Therefore, the question arises which mechanism(s) mediates natriuresis and suppression of renin in response to an acute modest sodium load. It seems likely that the MD mechanism is a major determinant of the renin release in response to an acute modest sodium load. Apart from the MD mechanism, a currently unknown mediator may be involved in the salt-induced changes of the renin system.

Taken together, sodium balance is an important regulatory mechanism for the activity of the renin system. It is currently unclear which mechanism or mediator is principally responsible for the salt-dependent regulation of renin synthesis and release, especially during chronic alterations in salt intake. The existence of such a humoral factor remains elusive.

C. Hormones, Autacoids, and Other Mediators

A large variety of biological agents are known to influence renin synthesis and/or renin release. Many of these are
of major relevance, and an established role in renin synthesis and/or secretion has been demonstrated; conversely, for others, there is only evidence on the cellular or isolated organ level or when given exogenously. These biological agents include classical hormones, autacoids, vitamins, cytokines, or the metabolite succinate (see Table 2). The role of cytokines and vitamins is described in section II, and the role of rather locally acting mediators, such as adenosine, norepinephrine, NO, endothelins, and prostanoids, has been described in section IV. In addition, the review by Hackenthal et al. (293) provides a detailed overview about the influence of angiotensin, antidiuretic hormone (vasopressin), prosta-glandins and leukotrienes, dopamine, ANP, kallikrein and kinins, vasoactive intestinal polypeptide, histamine, platelet-

<table>
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<td>Stimulation</td>
<td>Pharmacological tool</td>
<td>144, 145, 207, 320, 367, 445-447, 560</td>
</tr>
<tr>
<td>Tumor necrosis factor-α</td>
<td>Inhibition</td>
<td>Pharmacological tool</td>
<td>880, 884</td>
</tr>
<tr>
<td>Vasoactive intestinal polypeptide</td>
<td>Not determined</td>
<td>Pharmacological tool; stimulation in isolated superfused glomeruli</td>
<td>109, 198, 697</td>
</tr>
<tr>
<td>Vasopressin</td>
<td>Inhibition</td>
<td>Modulator</td>
<td>28, 99, 278, 334, 485, 556, 759, 776, 902</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>Stimulation</td>
<td>Pharmacological tool</td>
<td>796</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>Inhibition</td>
<td>Modulator</td>
<td>516, 980</td>
</tr>
</tbody>
</table>
activating factor, acetylcholine, calcitonin gene-related peptide, and endothelin in the release and synthesis of renin. If new findings have since been reported, the role of these mediators has been updated within this section. Otherwise, we refer readers to the review by Hackenthal et al. (293). For an overview of the putative receptors on JG cells, please see Table 3. It should be noted that Table 3 contains findings reported from cell cultures (As4.1 cells, isolated JG cells, and human transfected JG cells) and from histological approaches, which do not always definitively allow the conclusion that a receptor is colocalized with the native renin-producing JG cells.

1. β-Adrenergic pathway

It is well known that the sympathetic nervous system participates in the release of renin via the β-adrenergic pathway (293), as outlined in detail in section IV C. JG cells contain β1-adrenergic receptors (81, 161). The systemic application of the β1-adrenoreceptor agonist isoproterenol by chronic infusion into rats, both time and dose dependently, stimulates the synthesis and secretion of renin (355, 598, 802). In addition, isoproterenol infusion increased renin mRNA expression and PRA in rats during normal and high salt intake, but not during low salt intake (355). Notably, COX-2-derived prostanoids do not seem to be involved in the isoproterenol-induced stimulation of the renin system (435, 569).

In agreement with the stimulatory effects of the β-adrenoreceptor agonist isoproterenol on basal renin secretion, chronic β-adrenoreceptor antagonism has been found to decrease basal renin synthesis and secretion (354, 987). However, β-adrenoreceptor antagonism did not influence the increase in renin synthesis and secretion due to low salt intake (987), ANG II type 1 receptor blockade (924), or the combination of low salt intake with ACE inhibition (342), but it did attenuate the stimulation of the renin system in response to chronic stress (9), acute angiotensin AT1 receptor blockade (299, 662), and hypotensive hemorrhage (354). Additionally, oxytocin-induced renin release seems to be mediated via activation of the β-adrenergic system (360), whereas thyroid hormone stimulates the renin system independent of the sympathetic nervous system (447).

In summary, the sympathetic nervous system provides one of the most powerful background stimuli for renin synthesis and secretion. Its relevance as a regulatory factor of the renin system appears to be limited.

2. ANG II

Circulating ANG II functions in a direct negative-feedback loop (“short negative-feedback loop”) to inhibit renin synthesis and secretion (293). Numerous in vivo studies have shown that inhibiting the effects of ANG II

### Table 3. Expression of receptors on juxtaglomerular cells

<table>
<thead>
<tr>
<th>Mediator</th>
<th>Receptor</th>
<th>Species</th>
<th>Level of Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine A1</td>
<td>A1</td>
<td>Rat, rabbit</td>
<td>mRNA, functional (941, 943)</td>
</tr>
<tr>
<td>Aldosterone</td>
<td>MR</td>
<td>Mouse</td>
<td>mRNA, functional (444)</td>
</tr>
<tr>
<td>Angiotensin AT1</td>
<td>AT1</td>
<td>Human, rat, mouse, monkey</td>
<td>mRNA, protein (258, 267, 316, 365, 437, 677, 718, 893, 991, 992)</td>
</tr>
<tr>
<td>ANP</td>
<td>NPR1</td>
<td>Rat</td>
<td>Protein, functional (475, 622)</td>
</tr>
<tr>
<td>Bradykinin</td>
<td>B2</td>
<td>Human</td>
<td>mRNA, functional (696, 820)</td>
</tr>
<tr>
<td>Calcium</td>
<td>CaR</td>
<td>Mouse, rat</td>
<td>mRNA, protein, functional (553, 653)</td>
</tr>
<tr>
<td>Catecholamines</td>
<td>β1</td>
<td>Human, rat, dog</td>
<td>mRNA, protein, functional (81, 161, 327, 513, 831, 935)</td>
</tr>
<tr>
<td>CGRP, ADM</td>
<td>CALCRL</td>
<td>Human</td>
<td>Protein (297)</td>
</tr>
<tr>
<td>Dopamine D1, D5</td>
<td></td>
<td>mRNA, protein functional (18, 19, 476, 636, 963)</td>
<td></td>
</tr>
<tr>
<td>Endothelin</td>
<td>ETα, β</td>
<td>Mouse, rat</td>
<td>mRNA, functional (737, 948)</td>
</tr>
<tr>
<td>EGF</td>
<td>EGF-R</td>
<td>Rat</td>
<td>Protein, functional (219, 463, 727, 948)</td>
</tr>
<tr>
<td>Glucocorticoid</td>
<td>GR</td>
<td>Mouse</td>
<td>Protein (888)</td>
</tr>
<tr>
<td>Histamine H1</td>
<td>H1</td>
<td>Human</td>
<td>Functional (696)</td>
</tr>
<tr>
<td>Neuropeptide Y</td>
<td>Y1</td>
<td>Human</td>
<td>mRNA (949)</td>
</tr>
<tr>
<td>Neutrophins</td>
<td>trkB</td>
<td>Mouse</td>
<td>Protein (257)</td>
</tr>
<tr>
<td>Oxysterols LXRα</td>
<td>LXRα</td>
<td>Mouse, human</td>
<td>mRNA, protein, functional (509, 851)</td>
</tr>
<tr>
<td>Oxytocin OT</td>
<td>OT</td>
<td>Human</td>
<td>Protein (35)</td>
</tr>
<tr>
<td>PACAP PAC1</td>
<td>PAC1</td>
<td>Mouse</td>
<td>Functional (322)</td>
</tr>
<tr>
<td>Prostacyclin IP</td>
<td>IP</td>
<td>Rat</td>
<td>Functional (241, 401)</td>
</tr>
<tr>
<td>TGF-β</td>
<td>TGF-βII</td>
<td>Rat</td>
<td>Protein (620)</td>
</tr>
<tr>
<td>Thyroid hormone TR</td>
<td>TR</td>
<td>Rat</td>
<td>Protein (545)</td>
</tr>
<tr>
<td>Vasopressin VT1a</td>
<td>VT1a</td>
<td>Rabbit, mouse, rat, human</td>
<td>mRNA, protein (35, 36, 672)</td>
</tr>
</tbody>
</table>

CGRP, calcitonin gene-related peptide; ADM, adrenomedullin; ANP, atrial natriuretic peptide; EGF, epidermal growth factor; PACAP, pituitary adenylate cyclase-activating polypeptide; PGE2, prostaglandin E2; TGF-β, transforming growth factor-β. Nomenclature is in accordance with Alexander et al. (14). Reference numbers are given in parentheses.
with ANG II-AT₁ receptor antagonists or reducing the level of ANG II with ACE inhibitors causes a strong increase in renin synthesis and release (127, 346). Likewise, the exogenous infusion of ANG II decreases renin synthesis and renin release (775), suggesting that ANG II may exert direct negative effects on this system. Further support derives from mice lacking AT₁a receptors, which have an increased number of renin-positive JG cells (647). Since changes in the systemic ANG II concentrations, as well as alterations in the AT₁ receptor density, modulate the systemic blood pressure, an indirect effect of ANG II on renin synthesis and secretion via the systemic blood pressure cannot be excluded. However, it has also been found that nonpressor doses of ANG II inhibit renin secretion, suggesting a blood pressure-independent inhibitory effect of ANG II on renin release (181, 293). Further support for a pressure-independent effect of ANG II derives from the observation that ANG II inhibits renin secretion from isolated perfused kidneys and from kidney slices (616, 904). Such an inhibitory effect of ANG II on renin is in accordance with the existence of AT₁ receptors on the JG cells (Table 3). ANG II has also been found to decrease the COX-2 expression in MD cells via AT₁ receptors at lower concentrations than needed for systemic hemodynamic actions (984). Thus an inhibitory effect of ANG II on renin synthesis and renin release via a suppression of COX-2-derived prostanoids from MD cells may also be conceivable. Nevertheless, since JG cells are equipped with ANG II AT₁ receptors and because ANG II has been shown to decrease renin synthesis and release in As4.1 cells (105, 606), a direct negative effect of ANG II on renin release and renin synthesis can be postulated. In addition, it has been demonstrated that renal nerves are not essential for the ANG II negative-feedback loop (924), and this feedback loop is also relevant in various conditions of stimulated or reduced renin synthesis and secretion (802). However, a recent study in chimeric mice carrying a regional null mutation of the AT₁a receptor questions the direct effect of ANG II on the JG cells. In this report, the JGA of the AT₁a receptor-deficient mice were enlarged with intense expression of renin. In the chimeric mice, the changes in the JGA were proportional to the degree of chimerism, but the degree of JGA hypertrophy/hyperplasia and the expression of renin were unaltered in AT₁a expressing and AT₁a-deficient JG cells (568). More recently, renal cross-transplantation has been combined with gene knockout technology. The AT₁a receptor-deficient mice had increased renin mRNA and a lower blood pressure than wild-type controls. Mice that expressed the AT₁a receptor only in the kidney or only in extrarenal tissues had similar blood pressures; these were lower than that of wild-type controls, but they were higher than that of the complete AT₁a-deficient mice. Renin mRNA levels in renal or extrarenal knockouts were similar to wild-type controls but were significantly lower than in complete AT₁a-deficient mice (173). These data therefore suggest that the renal AT₁a receptors are key determinants of blood pressure, but they may not be of major importance for the regulation of renin expression. Thus these two recent studies suggest that the regulation of renin synthesis and release by ANG II is indirectly mediated, likely via changes in systemic blood pressure. In addition to the effect of circulating ANG II on the renal renin synthesis and secretion, ANG II in the brain may also have an inhibitory effect on the renin release. The central administration of ANG II decreases renin secretion, and the central administration of losartan increases renin secretion (387, 573, 942, 968). This effect on renal renin secretion is likely due to a reduction in the renal sympathetic nerve activity (574). Moreover, it should be kept in mind that ANG II facilitates the release of norepinephrine from the renal sympathetic nerve terminals (193).

In summary, ANG II inhibits renin release and synthesis through a direct short negative-feedback loop and also via indirect mechanisms.

3. ANP

The 28-amino acid peptide ANP is mainly released by the cardiac myocytes of the atrium in response to atrial stretch. The cardiovascular and renal effects of ANP (hypotension, natriuresis, and diuresis) counteract the effects of ANG II (837). ANP can modulate the renin release through direct and indirect mechanisms. The activation of the natriuretic peptide receptor-1 (NPR1) can directly inhibit the renin release by increasing the cGMP formation. Although it is generally believed that the tubular action of ANP is predominantly exerted in the more distal segments of the nephron, ANP may also indirectly reduce the renin release via a possible effect on the proximal tubular salt reabsorption (314), which may lead to an increased NaCl load at the MD. In contrast, ANP can indirectly stimulate renin release by lowering the blood pressure. Indeed, somewhat conflicting data have been reported regarding the role of ANP in renin release, although most of these studies, including in vitro studies using isolated JG cells, suggest that ANP is an inhibitor of renin release (293). More recent studies found that the incubation of rat renal cortical slices with or intravenous infusion of ANP into humans did not affect the renin release (377), whereas the intrarenal and intravenous infusion of ANP in dogs stimulated and inhibited renin release, respectively (213). ANP-deficient mice have lower renal renin mRNA levels but increased plasma renin concentrations and are hypertensive (576, 638). In addition, the genetic deletion of the NPR1 receptor leads to hypertension, which is paralleled by a reduction in the plasma renin concentration and in renal renin expression (797). The increase in systemic blood pressure observed...
in the ANP- and NPR1-knockout mice may indirectly mediate the inhibition of the renal renin gene expression.

Taken together, an inhibitory effect of ANP on the renin secretion at the JG cell level has been demonstrated (411, 475). The inhibition of renin release by ANP may be modulated by the cardiovascular and renal effects of ANP in vivo.

4. Vasopressin

Arginine vasopressin (AVP), also known as antidiuretic hormone (ADH), influences a wide variety of physiological functions; more specifically, the induction of antidiuresis and vasoconstriction are considered the most important effects of AVP. AVP acts on three G protein-coupled receptors, termed the vasopressin V1a, V1b, and V2 receptors (44, 891). In the kidney, the V1a receptors, which mediate the vasoconstrictor effect of AVP, are localized in the renal vasculature, the JG apparatus, the MD cells, the connecting tubule, and the cortical collecting duct (28, 35, 672). The V2 receptor expression, which mediates the antidiuretic effect of AVP, has been found in the TAL, the MD cells, the distal convoluted tubule, the connecting tubule, and the collecting duct (614, 672). The renal expression of the V1b receptor has also been reported, but the precise localization and function of this receptor are unclear (531, 595).

AVP also modulates renin release, and the inhibitory effect of AVP on renin release was favored by older investigations (293). Among other findings, AVP has been shown to decrease renin release from primary cultures of renin-producing JG cells (485). Because vasopressin V1a shown to decrease renin release from primary cultures of investigations (293). Among other findings, AVP has been reported, but the precise localization and function of this receptor are unclear (531, 595).

AVP also modulates renin release, and the inhibitory effect of AVP on renin release was favored by older investigations (293). Among other findings, AVP has been shown to decrease renin release from primary cultures of renin-producing JG cells (485). Because vasopressin V1a but not V2 receptor expression has been described for the afferent arteriole (35, 614, 672), one may assume that this inhibitory effect could be mediated via vasopressin V1a receptors (776). In line with this assumption, it has been found that the preferential V1a agonist terlipressin decreased the plasma renin concentration in patients with hepatorenal syndrome (294, 897). However, terlipressin also improved the renal function and blood pressure (605, 897). Contrary to these findings, it was recently reported that PRA and renin mRNA were lower in the vasopressin V1a receptor-deficient mice compared with wild types (28). The decrease in the renin system in these mice was paralleled by a decreased expression of nNOS and COX-2 in MD cells and alterations in systemic and renal hemodynamics, such as lower blood volume, hypotension, decreased renal blood flow, and GFR (28, 459). Since the expression of vasopressin V1a receptors has been found in MD cells (28), AVP may also influence renin release via a MD-dependent mechanism. AVP may also modulate the renin system via the vasopressin V2 receptors. It has been found that the infusion of the vasopressin V2 receptor agonist desmopressin (dDAVP) decreases blood pressure and increases the PRA in humans (47, 72). Subsequently, it was reported that the stimulatory effect on renin release was inhibited by the COX inhibitor indomethacin, independent of the blood pressure-lowering effect of dDAVP (319). However, the infusion of dDAVP into humans and animals also increases the renal blood flow and GFR, probably due to a vasodilatation of the afferent arteriole (86, 231, 619, 701, 849). A stimulatory effect of dDAVP on renin release has been reported for the isolated perfused rat kidney and from rabbit renal cortical slices (319, 762), and chronic infusion of dDAVP has been shown to increase renin mRNA levels in Brattleboro rats, which lack AVP (20). Although these data support a stimulatory role for the vasopressin V2 receptor in renin release, it has recently been found that renin mRNA is also increased in vasopressin V2 receptor-deficient mice (759), which exhibit a clear renal phenotype (981). Furthermore, the vasopressin V2 receptor antagonist tolvaptan did not change the plasma renin activity in rats and humans (800, 801, 911).

Due to its complex cardiovascular and renal effects in vivo, the role of vasopressin and the respective receptors in the control of renin release is still controversial and needs further evaluation. One could assume that AVP inhibits renin release under some conditions via the activation of the vasopressin V1a receptors, but it may also increase renin secretion via the activation of V2 receptors.

5. Oxytocin

In addition to its well-known effects on uterine contraction and milk ejection, oxytocin has been shown to influence renal function, such that oxytocin increases the GFR and natriuresis in rats (167). Oxytocin activates a single G protein-coupled receptor, termed the OT receptor (268). Because the expression of OT receptors has been found in MD cells (35, 658, 828), a role for oxytocin in renin release was presumed. It has been reported that oxytocin increases the plasma renin concentration in rats and that this increase may be independent of the oxytocin-induced natriuresis (810). Subsequently, it was found that oxytocin-induced renin secretion was inhibited by β-adrenergic receptor antagonism (360), but not by renal denervation (530). Therefore, circulating catecholamines may be involved in renin release in response to oxytocin. Because oxytocin is released in response to hypotension and hypovolemia, it has been speculated that oxytocin could stimulate renin release under these conditions. The OT receptor antagonist atosiban attenuates the rise in PRA and heart rate in response to hydralazine or diazoxide, without affecting the blood pressure-lowering effect of these two vasodilators (361). In contrast to these findings, the intravenous infusion of oxytocin decreased urine flow and reduced sodium excretion and PRA in humans (716). The opposite
finding in humans may be due to a different expression of the renal OT receptors (35).

In summary, oxytocin stimulates renin secretion in laboratory animals via a β-adrenergic-dependent mechanism. The stimulatory effect may be species dependent, probably due to a different expression of renal OT receptors.

6. Aldosterone

The stimulation of renin release increases the plasma levels of ANG II, which subsequently stimulates the secretion of aldosterone from the adrenal glands (821). The stimulation of angiotensin II and aldosterone formation increases the sodium reabsorption and consequently increases the body sodium content, which, in turn, inhibits the renin gene expression (293, 488, 821). Recently, it was shown that JG and As4.1 cells express mineralocorticoid receptors; additionally, aldosterone increased renin mRNA levels in primary cultures of mouse JG cells pre-stimulated with isoproterenol, but it had no effect on the exocytosis of stored renin (444). Conversely, mineralocorticoid receptor- and aldosterone synthase-deficient mice have a strong increase in plasma renin concentration, probably due to a sodium deficit caused by renal sodium wasting (71, 555).

These data, therefore, suggest that aldosterone exerts a direct positive effect on renin gene expression at the cellular level, probably by stabilizing renin mRNA. However, the in vivo relevance of this finding remains unclear, and therefore, further investigation is necessary. Moreover, the in vivo effect of aldosterone may be indirectly due to changes in the body sodium content, extracellular volume, or blood pressure.

7. Glucocorticoids

It is well known that the administration of glucocorticoids increases the GFR via a vasodilatation of afferent and efferent arterioles and increases the renal blood flow and blood pressure (56, 82, 183, 950). Several findings suggest that glucocorticoids also regulate renin concentration and renin release. In sheep, a birth peak of cortisol seems to be responsible for the parallel activation of renin (231a). Furthermore, glucocorticoids have been found to directly activate renin synthesis in JG cells (444). Glucocorticoids act on glucocorticoid receptors (GR), which have been mainly localized in the glomeruli, proximal tubule, and TAL segments, including the MD cells (549, 879a).

In addition, cortisol can bind and activate the mineralocorticoid receptor (MR). However, the 11β-hydroxy-steroid dehydrogenase type 2 enzyme (11β-HSD2) is mostly coexpressed with the MR and inactivates cortisol to cortisone, which is not able to bind to the MR. Since afferent arterioles, isolated JG cells, and As4.1 cells express 11β-HSD2, a direct mineralocorticoid effect of the glucocorticoids on renin release and synthesis may be unlikely (444, 895a). In addition, the glucocorticoids may indirectly induce renin release via a possible effect on the proximal tubular salt reabsorption, which may lead to a decreased NaCl load at the MD (634).

On the other hand, it has been found that cortisol can reduce the plasma renin activity in adult ewes (425). The treatment of rats with dexamethasone did not alter the PRA, although it did strongly increase the blood pressure (838). Furthermore, COX-2 expression in the TAL and in the MD cells is suppressed by endogenous glucocorticoids (549, 915, 984).

Taken together, a direct stimulatory effect of glucocorticoids on renin synthesis has been shown in vitro. The stimulatory effect of glucocorticoids on renin release and synthesis may be modulated by an increase in blood pressure and by the suppression of the MD COX-2-derived prostanoids in vivo.

8. Thyroid hormone

Several in vivo and in vitro studies have suggested that the thyroid hormone influences renin synthesis and secretion. Hyperthyroidism in response to l-thyroxine or triiodothyronine (T₃) treatment increased, while hypothyroidism due to thyroidectomy or methimazole treatment reduced, the PRA and renin mRNA in rats and fetal sheep (144, 145, 207, 446, 560). Moreover, the stimulation of renin synthesis and renin secretion by thyroid hormone seems to be independent of sympathetic nerve activity, since the chemical sympathetic denervation with 6-hydroxydopamine did not affect the stimulatory effect of the thyroid hormone on renin synthesis and renin secretion (447). In vitro, thyroid hormone enhanced renin secretion from rat kidney slices and from primary cultures of JG cells (320, 367). In addition, renin mRNA was found to be increased in primary cultures of JG cells by T₃ and in rats in response to l-thyroxine (367, 446). These observations suggest a possible direct effect of thyroid hormone on the activity of the renin system. Subsequently, it was found that thyroid hormone induces the promoter activity of the human renin gene through thyroid hormone response element-dependent mechanisms in Calu-6 cells (445).

9. Sex steroids: testosterone, estrogen, and progesterone

Various studies have indicated that blood pressure is higher in men than in women and that blood pressure increases after menopause in women (107, 430, 565, 823). In addition, the increase in blood pressure starts with puberty, suggesting a role of the sex steroids (39, 317). Sex-dependent differences in blood pressure are also known for several animal models (289, 351, 431, 719). Male spontaneously hypertensive rats (SHRs), for example, have higher blood pressure than females, and orchi-
pectomy, but not ovariectomy, attenuated the increase in systolic blood pressure (148, 217). Since the renin-angiotensin system is a major system for the long-term control of blood pressure and salt and water homeostasis, gender-dependent differences in the synthesis and release of renin from the kidney may be involved in the sex-dependent blood pressure regulation. It has been found that the PRA is higher in men than in women and that it increases in postmenopausal women (395, 774). The chronic treatment of gonadectomized SHRs with testosterone increases blood pressure and PRA in male and female SHRs, and PRA decreases with castration in male rats (149, 217). Furthermore, the administration of testosterone with dihydrotestosterone (DHT), act on androgen receptors, which are expressed in mammalian kidneys (739). Although the precise localization of these receptors is not entirely clear, it seems likely that the androgen receptors are mainly expressed in proximal tubular cells where testosterone enhances the salt and water reabsorption, likely via an increased expression of the proximal tubular transporter NHE3 (705, 739). There is no evidence for the expression of testosterone receptors in JG cells or MD cells. In line with this, testosterone did not alter the levels of renin mRNA in the As4.1 cell line (444). Otherwise, no effect, or a decrease in renal renin mRNA expression, has also been reported for mice (132, 700, 928). As a result, testosterone may indirectly influence the renal system, likely via an increased absorption of salt and water at the proximal tubule, which may lead to a decreased NaCl load at the MD. This effect may be modulated by an increased extracellular volume and increased blood pressure in vivo.

In contrast to androgens, estrogens tend to have antihypertensive effects, mainly via the control of local blood flow and peripheral resistance due to alterations in the release of the endothelial mediators (359, 551). Interactions between estrogens and renin have also been reported. Several studies support a stimulatory effect of estrogen on renin release. Increases in endogenous estrogen levels are associated with increased PRA in healthy women, for example, the PRA increases during the luteal phase of the menstrual cycle, when plasma estrogen levels are highest (137, 153, 787). Furthermore, estrogen administration in animals increased the PRA, which was paralleled by a decrease in blood pressure (550, 551), and estrogen replacement increased the PRA in cynomolgus monkeys (89). On the other hand, it has also been found that, after menopause, blood pressure and PRA increase in women, and the renin levels in women with estrogen replacement therapy were lower than in those without estrogen therapy (178, 774). In animals, estrogen administration reduced the PRA and blood pressure in ovariectomized mRen2(2) Lewis rats (138). The mechanism for a possible modulatory effect of estrogen on the renin secretion is not understood. Moreover, the information on the renal distribution of the estrogen receptor is rather limited (739), and no expression has been reported for the MD cells (698, 829). Whether the JG cells contain estrogen receptors has yet to be determined. Since estradiol did not alter the renin mRNA in the As4.1 cell line (444), it seems likely that indirect, rather than direct, mechanisms may be involved in the modulation of renin secretion by estrogens. In this regard, estrogen has been reported, for example, 1) to increase the renal oxytocin receptor gene expression (659), 2) to attenuate the response of ANG II on the AT1 receptors (34, 252, 628, 804), 3) to increase the renal NO synthesis (55), 4) to modulate the bradykinin B2-receptor gene expression and function (547), and 5) to inhibit the sympathetic nervous system (187, 234, 523). In addition, during low estrogen levels, the effect of androgens on renin release may be more apparent (719). Taken together, the influence of estrogen on the renal release of renin remains controversial mainly due to the complex influences on the cardiovascular functions in vivo.

Progestrone acts on progesterone receptors, but the precise renal localization of these receptors is not clear (739). With regard to renin synthesis and renin release, it has been reported that the administration of progesterone increases PRA in humans (642). Furthermore, the increase in PRA during the menstrual cycle was attenuated by a synthetic progestrone (641). In addition to an action on the progesterone receptors, progesterone can also act as a mineralocorticoid receptor antagonist (98). Thus the natriuretic and blood pressure-lowering actions of progesterone have mainly been related to this antagonistic effect (136, 642, 707). Therefore, progesterone may also attenuate the action of aldosterone on renin synthesis and renin release. In addition, progesterone can be metabolized to testosterone and dihydrotestosterone in the kidney and may therefore have androgenic effects, probably also on renin release (706).

10. Adipokines: leptin and adiponectin

Obesity is a major risk factor for the development of hypertension (232, 301). The activation of the sympathetic nervous system and of the RAAS, as well as the endothelial and renal dysfunctions, has been implicated in the association between obesity and hypertension (710). In the past few years, it has become apparent that adipose tissue is not only a reservoir for energy, but it is also an endocrine organ, which secretes a variety of bioactive
peptides, known as adipokines (427). Among these, leptin and adiponectin in particular have been suggested to be involved in the development of obesity-induced hypertension and, therefore, also for the activation of the RAAS in obesity.

Leptin, the product of the obesity gene, is a circulating peptide that is primarily synthesized and secreted by the adipocytes. Originally, it was described as an adipostat that controls energy balance (532, 585). Later on, it was observed that leptin also influences the cardiovascular and renal functions and that the increased levels of leptin in obesity may contribute to obesity-induced hypertension. It has been found, for example, that the infusion of leptin activates sympathetic nerve activity and increases the mean arterial blood pressure, heart rate, urine volume, and sodium excretion in rats (323, 389, 793, 914). Reports of a positive correlation between leptin and PRA in obesity and essential hypertension in humans suggest a potential role for leptin in renin release (5, 836, 894), but up to now, no direct effect of leptin on the release of renin from the kidney has been reported. In contrast, the carotid artery or intravenous infusion of leptin did not alter PRA in rats (793, 914). However, leptin may indirectly induce renin release via a central activation of the renal sympathetic nervous system (120, 323, 561, 711, 712, 910). In accordance with this, it has been reported that renal denervation inhibits the rise in PRA, at least in response to a high-fat diet in dogs (421).

Adipocytes also secrete adiponectin, a peptide that has been described to enhance insulin sensitivity and prevent arteriosclerosis (644, 964). The plasma levels of adiponectin have been found to be lower during obesity and in hypertensive men, and they are negatively correlated with blood pressure (33, 385, 509). Adiponectin has been found to ameliorate obesity-related hypertension in mice (643) and to decrease blood pressure and renal sympathetic nerve activity in rats (856). Furthermore, the inhibition of the RAAS increases and infusion of ANG II decreases plasma adiponectin levels (249, 509). It is unknown whether there is a direct effect of adiponectin on renal renin release. However, since adiponectin decreases the renal sympathetic nerve activity, it may indirectly influence renal renin release.

11. Growth hormone and insulin-like growth factor I

The administration of growth hormone (GH) into healthy men increases the PRA without changes in blood pressure or decreases in the sodium and water excretion (340, 590). In line with these findings, the inhibition of the RAAS prevented the GH-induced fluid retention in humans (592). Furthermore, GH was found to increase the PRA in GH-deficient humans and rats (175, 247, 588, 689, 959). Consistent with a stimulatory effect of GH on renin release, it has been found that a growth hormone receptor deficiency results in reduced plasma renin concentration despite a decrease in blood pressure (212). The stimulatory effect of GH on renin release may be due to an increased formation of COX-derived prostanoids (310).

It has been suggested that several effects of GH are at least partly mediated by insulin-like growth factor I (IGF-I) (501). Indeed, it has been found that IGF-I increases PRA in rats and fetal sheep and stimulates the secretion of renin from renal cortical slices (392, 408, 563, 564), but the precise role of IGF-I for GH-induced renin release is unknown. On the other hand, no effect of GH on the PRA and renin mRNA or on the secretion of renin from primary cultures of JG cells have been reported (51, 333, 413, 468).

In summary, it can be concluded that GH stimulates renin synthesis. Since GH did not induce renin release from the JG cells, the effect of GH on renin secretion may be rather indirect and may depend on the formation of COX-derived prostanoids.

12. Prostanoids

PGI2 and PGE2 are direct stimulators of renin synthesis and secretion that most likely function via enhanced cAMP formation (293). Due to their short half-life, it is still unclear whether extrarenal-derived PGI2 and PGE2 could serve as stimulators of renin secretion and synthesis. Therefore, PGI2 and PGE2 have been suggested to function as local, rather than systemic, regulators of these processes (see sect. iv, A1 and B4). However, since PGI2 is not completely metabolized when it passes through the pulmonary system and since the half-life of PGI2 is between 30 s and several minutes (541, 594, 906), it may be theoretically possible that systemic-derived PGI2 is of relevance for renal renin release under certain circumstances, such as furosemide treatment or sepsis (93, 345, 518).

13. Dopamine

The neurotransmitter dopamine modulates several physiological functions within the kidney, including renin release and sodium excretion. Dopamine can directly stimulate renin release via the activation of the D1-like receptors upon the dopaminergic innervation of the vascular pole of the glomerulus. Increases in the systemic dopamine concentration can also indirectly affect renin release by lowering blood pressure. In addition, dopamine can negatively influence renin release and synthesis via its effect on the proximal tubular sodium reabsorption. This effect is important because the main source of dopamine within the kidney is the proximal tubule itself. Here, the glomerular filtered dopamine precursor L-DOPA is reabsorbed and then converted into dopamine by the action of aromatic L-amino acid decarboxylase, which is strongly expressed in proximal tubular cells. Therefore, dopamine
signaling leads to an increase in the tubular sodium chloride concentration at the MD (293).

Most investigators have observed a stimulatory effect of dopamine on the renin secretion at the level of kidney slices, isolated perfused kidneys, and in vivo (293). Two recent studies further support a stimulatory role for dopamine in renin synthesis and secretion. The authors reported that dopamine induces renin release from rat JG cells (963) and isolated glomeruli via DA1 receptors (307). The effect of dopamine on renin release was also recently investigated in mice carrying a genetic deletion of catechol-O-methyltransferase (COMT), the main enzyme in the renal metabolism of dopamine. These data suggest that dopamine inhibits renin release and synthesis indirectly via increased NaCl load at the MD during normovolemia or hypovolemia; moreover, the data suggest that dopamine directly stimulates renin release in situations of volume expansion (985).

In summary, dopamine can directly stimulate renin release and synthesis, most likely via DA1 receptors at the JG cell level. However, the direct stimulatory effect of dopamine on renin release and synthesis is largely compensated for by the indirect inhibitory cardiovascular and renal effects of dopamine in vivo.

### 14. Succinate/GPR91

The orphan G protein-coupled receptor GPR91 has recently been shown to function as a receptor for succinic acid. GPR91 mRNA was found to be expressed predominantly in the kidney and, at lower levels, in the liver and spleen. The in situ hybridization revealed GPR91 expression in the proximal tubules, distal tubules, and the JGA of the mouse kidney. The intravenous injection of succinate increased PRA in Sprague-Dawley rats (324), confirming a report that examined the rate of renin release from viable JG cells during the superfusion of isolated rat glomeruli (53). Moreover, blood pressure also increased in response to succinate in a dose-dependent manner. The increase in blood pressure was eliminated in the bilateral nephrectomized animals, attenuated in the animals pretreated with the ACE inhibitor captopril, and absent in the nephrectomized animals, attenuated in the animals pre-treated with the ACE inhibitor ramipril, although the PRC was lower in PAC1 receptor knockout mice fed a low- or high-salt diet, compared with the wild-type mice, and in response to treatment with the ACE inhibitor ramipril, although the principal regulation was not attenuated. Therefore, it may be inferred that PACAP is a tonic enhancer of the renin system in vivo via the activation of adenylyl cyclases (322).

### 15. PACAP

PACAP is involved in the regulation of the cardiovascular and central nervous system (909). PACAP was recently shown to dose-dependently stimulate renin secretion from isolated perfused kidneys of rats and from primary cultures of renin-producing JG cells. The PACAP-induced renin release from isolated kidneys was attenuated in pituitary adenylate cyclase 1 (PAC1) receptor-deficient mice, which also exhibited lower basal PRC and lower blood pressure. Moreover, the PRC was lower in PAC1 receptor knockout mice fed a low- or high-salt diet, compared with the wild-type mice, and in response to treatment with the ACE inhibitor ramipril, although the principal regulation was not attenuated. Therefore, it may be inferred that PACAP is a tonic enhancer of the renin system in vivo via the activation of adenylyl cyclases (322).
Renal tissue levels of angiotensin I were found to be diminished in these B2 receptor knockout mice, while the ANG II levels were reportedly unchanged. In addition, these authors observed decreased renal COX-2 gene expression in the B2 receptor knockout mice (374). They concluded that the B2 receptor-dependent regulation of COX-2 participates in the basal control of renin gene expression. With regard to the lower renin mRNA expression, a similar finding was reported for the newborn and adult B2 receptor-deficient mice (976). In addition, kidney renin mRNA levels did not differ between the B2 receptor-deficient mice and wild-type controls during normal and chronic high salt intake (134). The role of the bradykinin B2 receptor was further investigated in mice overexpressing the human version of the protein. These hypotensive mice exhibited enhanced renal function with increased urinary excretion of nitrite/nitrate cGMP, cAMP, and kinin. However, the renal renin mRNA abundance was not altered in these mice (933).

The treatment of rats with HOE-140 (Icatibant), a B2 receptor antagonist, or the kallikrein inhibitor aprotinin did not alter basal renal renin mRNA abundance (215, 977). The intravenous infusion of HOE-140 decreased the basal PRA in rabbits independent of any changes in blood pressure, but it did not attenuate the furosemide-stimulated elevation in the PRA (155). HOE-140 did not alter the basal PRA or the furosemide- or valsartan-induced PRA changes in humans (504, 612). However, HOE-140 inhibited the increase in PRA and the decline in blood pressure observed in response to captopril in humans (251). In contrast, HOE-140 did not alter the renal or enalapril-induced PRA and PRC changes in rats with passive Heymann nephritis, although HOE-140 attenuated the decreased blood pressure effect of enalapril (363). Moreover, the DOCA-suppressed PRA was not altered by HOE-140 in rats (546).

In summary, the precise role of bradykinin in renin synthesis and secretion is still controversial, probably because of the complex nature of the cardiovascular and renal effects of bradykinin in vivo.

17. Adrenomedullin

Adrenomedullin (ADM) is a peptide with renal and cardiovascular activities, and the immunoreactivity of the proadrenomedullin NH2-terminal 20-amino acid peptide has been localized in the JG cells (536). The plasma levels of ADM have been shown to significantly correlate with the PRA in patients with heart failure (422) and cirrhosis (450), suggesting a potential role for ADM in the secretion of renin. Accordingly, the intravenous administration of ADM in rabbits and sheep caused a decrease in the mean arterial blood pressure, which was paralleled by increases in the heart rate and PRA (141, 247, 673). In addition, ADM infusion into healthy volunteers or patients with heart failure, essential hypertension, or chronic renal impairment increased the PRA (495, 496, 572, 892). This effect was not observed in patients with pulmonary hypertension (618). Subsequent studies demonstrated that the renin release from isolated perfused rat kidneys is dose-dependently increased by ADM (397) and that ADM stimulates renin release and renin mRNA synthesis in mouse JG cells, suggesting a direct effect in renin synthesis (397). On the other hand, it has been reported that the intrarenal infusion of ADM in dogs increased the renal blood flow, urine flow, and urinary excretion of sodium and potassium without alterations in blood pressure, heart rate, or PRA (209, 409). Similarly, ADM infusion attenuated the rise in PRA and blood pressure due to left renal artery clipping in rats (428). In addition, a negative correlation was found between the plasma ADM concentrations and PRA in patients with chronic glomerulonephritis (438). Recently, it was reported that the infusion of ADM-2, a new member of the calcitonin gene-related peptide superfamily, increased the PRA in normal conscious sheep (140). Taken together, a direct stimulatory effect of ADM on renin secretion at the JG cell level has been shown, although this effect may be modulated by the cardiovascular and renal effects of ADM under certain in vivo circumstances. The possible stimulatory effect of ADM-2 warrants further investigation.

18. Neuropeptide Y

The vasoactive peptide neuropeptide Y (NPY) is coreleased with norepinephrine from sympathetic nerve terminals. In addition to the expression of the NPY receptor subtype Y1 in the collecting ducts and the loop of Henle, the Y1 receptor expression has been described for the JGA (949). Therefore, a possible role for NPY in the release of renin has been suggested. It has been shown that the infusion of NPY inhibited renin secretion in isolated perfused rat kidney and in animal models, which was paralleled by renal vasoconstriction (77, 168, 292). Furthermore, NPY was found to decrease the PRA in adrenalectomized rats (693), in postmyocardial infarction rats (982), in response to isoproterenol or epinephrine (10, 38, 221), and in two-kidney one-clip renal hypertensive rats (920). On the other hand, no changes in PRA in response to nonpressor doses of NPY have also been reported (37, 221, 684, 920, 982). In addition, no effect of NPY was observed on renin release from renal cortical slices (798). Furthermore, no renal phenotype has been reported in mouse models deficient in NPY or in Y1 receptors (953), although the administration of NPY has been found to decrease the renal blood flow and to induce diuresis and natriuresis (78).
Taken together, NPY may inhibit renin release under some experimental conditions or with pharmacological doses, but it does not seem to be involved in the physiological control of renin secretion.

V. ACTION OF RENIN

Most of the major known effects of the renin-angiotensin system (RAS) are related to the activity of angiotensin II (ANG II). Consequently, the end point of the renin-angiotensin cascade is generally assumed to be the generation of ANG II and related peptides. In this section, we briefly overview the data relevant to the signaling and function of the various angiotensin receptors, as reviewed in more detail elsewhere (575). In addition, we discuss data showing how angiotensin receptors are subject to regulatory processes, including changes in the angiotensin receptor expression, receptor desensitization/internalization, and dynamic interaction with angiotensin receptor-binding proteins. Furthermore, there is growing evidence that alternate peptides like ANG-(1–7) and their respective receptors broaden the functional range of the RAS. Finally, the action of renin is not restricted to its proteolytic activity and subsequent angiotensin generation; there is accumulating evidence that renin and/or pro-renin exert direct effects upon binding to renin/pro-renin receptors (Fig. 6).

A. AT₅ and AT₂ Receptors

Two receptor subtypes for ANG II have been characterized, AT₁ and AT₂. In rodents, AT₁ exists as two highly homologous subtypes, AT₁a and AT₁b. While AT₁a and AT₁b have similar functions under most conditions, AT₂a-mediated signaling differs considerably from AT₁-dependent signaling, as it partly antagonizes the effects of ANG II on AT₁ receptors.

1. AT₁, AT₁a, and AT₁b receptor function and signaling

AT₁ receptors mediate most of the known effects of ANG II. The AT₁ receptor, a 40-kDa protein consisting of 359 amino acids, is a member of the seven-transmembrane domain G protein-coupled receptor family. The AT₁ receptors typically couple to Gq complexes, resulting in the activation of downstream intracellular signaling pathways that lead to the activation of phospholipase C, phospholipase A₂, and phospholipase D (898). The activation of phospholipase C triggers the enhanced formation of IP₃ and diacylglycerol, promoting the subsequent release of calcium from intracellular stores and the activation of PKC, respectively. For a comprehensive review of the manifold intracellular signaling processes triggered upon the binding of ANG II to AT₁ receptors, see Reference 575. In most mammalian species, there is only one distinct type of AT₁ receptor. However, in rats and mice, two isoforms of AT₁ exist, AT₁a and AT₁b. They share high amino acid homology (>95%), and so far, no pharmacological tools have been available to specifically, or even preferentially, block one of the isoforms (280).

ANG II exerts myriad functions via AT₁, including but not limited to vasoconstriction, increased renal tubular salt reabsorption, stimulation of aldosterone release, and central effects like elicitation of thirst and vasopressin secretion. Under pathological conditions, ANG II was shown to promote vascular remodeling, cardiac hypertrophic remodeling, and extracellular matrix deposition (206).
In view of these multiple functions and considering the broad expression pattern of AT1 receptors in almost all organs, it is not surprising that mechanisms have evolved to regulate AT1 receptor function, both spatially and temporally. These mechanisms include 1) regulation of AT1 receptor transcription, 2) regulation of AT1 receptor surface expression via internalization and membrane recycling, and 3) modulation of AT1 receptor activity by accessory proteins.

2. Regulation of AT1 receptor transcription

The chronic elevation of ANG II levels in vitro has been reported to activate a feedback loop that results in the downregulation of the AT1 receptor expression (279, 284, 501). Unfortunately, the situation in vivo is less clear. During a low-salt diet, and by inference elevated ANG II levels, the downregulation of the AT1 receptor expression in the rat liver has been reported (150, 760). The regulation of AT1 expression in the kidney under a low-salt diet is spatially heterogeneous. AT1 expression was also found to be upregulated in the proximal tubule but downregulated in glomeruli (150). In the adrenal gland, AT1 expression was augmented during a low-salt diet (384, 506, 760). These diverse results clearly suggest that multiple factors affect AT1 receptor expression in response to a salt-restricted diet. In fact, AT1 expression was shown to be regulated by numerous factors other than ANG II. These include low-density lipoprotein (629), insulin (845), and progesterone (630); meanwhile, ANG II (284), NO (371), and estrogens (630) suppress AT1 expression. Thus modulation of AT1 expression may constitute a measure to locally adapt the sensitivity of target cells and organs when they are exposed to changes in systemic ANG II levels. For example, a salt-restricted diet, accompanied by enhanced systemic ANG II concentrations, stimulates various salt-conserving mechanisms, like aldosterone release and renal sodium reabsorption, but it has little, if any, effect on the vascular resistance. It remains to be determined to what extent the modulations of AT1 receptor expression are relevant for this apparent local fine-tuning of ANG II sensitivity.

3. Regulation of AT1 receptor surface expression by internalization and membrane recycling

AT1 receptors are subject to internalization and desensitization. The continuous cycle of receptor internalization and cell surface retrafficking was shown to be shifted towards internalization upon binding of ANG II to the AT1 receptor. As a consequence, the number of available receptors in the cell membrane was reduced, resulting in cell desensitization for ANG II (205, 279, 284). In addition, AT1 receptors located in the cell membrane are also subject to direct desensitization. Various members of the G protein-coupled receptor kinase family (GRK; Refs. 505, 529), including GRK2, GRK3, and GRK5, were found to be capable of phosphorylating serine and threonine residues located in the cytoplasmic domain of AT1 receptors. The phosphorylation of AT1 resulted in inactivation of the cell membrane-bound AT1 and promoted receptor internalization (412, 649).

In summary, the AT1 receptor internalization and surface trafficking permit regulation of the AT1 receptor surface expression and, consequently, influence the magnitude of local ANG II effects.

4. Modulation of AT1 receptor activity by accessory proteins

The set of molecular mechanisms that modulates AT1 receptor signaling also includes the interaction of AT1 with AT1-associated proteins. During the last few years, different proteins that interact with the COOH-terminal, intracellular tail of the AT1 protein have been identified. These AT1-associated proteins include negative and positive regulators of AT1 signaling. The AT1-associated protein Atrap (angiotensin receptor-associated protein) was identified as a negative regulator of AT1 (180), whereas ARAP1 (angiotensin receptor-associated protein 1 = angiopoietin-related protein 2; Ref. 286) and GLP (GEF-like protein) exert positive regulatory functions. ARAP1 overexpression in the proximal tubule of mice results in hypertension and kidney hypertrophy, suggesting that ARAP1 may function as a positive regulator of the AT1 receptors (285). The function of the fourth known AT1-associated protein, EP24.15 (metalloendopeptidase 24.15), remains unknown (799).

5. Atrap: a negative regulator of AT1 receptors?

The investigation of the function of AT1 receptor-associated proteins has only recently begun, with Atrap probably being the best characterized AT1 receptor accessory protein. Atrap is an 18-kDa protein with three putative transmembrane domains (534). It is expressed in various organs, with the highest expression levels observed in the kidney, heart, and testes; on the other hand, lower expression levels were found in the lung, liver, spleen, and brain (180). In the kidney, Atrap was detected by immunohistochemistry primarily in the tubular system, where it partially colocalized with the AT1 receptors. Little Atrap expression was detected in the renal vasculature, despite the marked expression of AT1 receptors, implying a specific function of Atrap in modulating the tubular effects of ANG II (893). The functional impact of the Atrap-AT1 interaction has been assessed in several studies. In vascular smooth muscle cells, the overexpression of Atrap resulted in enhanced internalization of the AT1 receptor upon exposure to ANG II (174). Increased AT1 receptor internalization was accompanied by marked...
inhibition of the angiotensin-induced proliferation of vascular smooth muscle cells (174). Similar results were obtained for cardiomyocytes, in which the prohypertrophic effect of ANG II was reduced during the coexpression of Atrap and paralleled by reduced surface expression of AT1. Once again, this indicates that Atrap promotes the internalization of AT1 receptors (855). It should be noted that the mutations in the COOH-terminal end of the AT1 receptor, the putative binding site of Atrap, result in a reduced internalization frequency of the AT1 receptor (166, 329). Thus far, it remains unclear whether the negative effect of Atrap on the AT1 signaling is merely due to a reduction of the AT1 surface localization or whether Atrap also interferes with the AT1-dependent intracellular signaling (517). A first attempt to address the in vivo function of Atrap was recently made via the generation of a transgenic mouse line that overexpresses Atrap under the control of the CMV promoter (Atrap-Tg) (657). Atrap mRNA expression in the heart, aorta, and femoral artery was increased three- to fourfold compared with the wildtype controls (657). The arterial blood pressure and heart rate were indistinguishable between Atrap-Tg and wildtype mice. However, the effect of cuff placement around the femoral artery as an experimental approach to assess vascular inflammation and remodeling was considerably ameliorated in Atrap-Tg mice. Similarly, the progression of heart hypertrophy after aortic banding was attenuated in Atrap-Tg mice compared with wild-type mice, indicating that the overall effect of Atrap is attenuation of the AT1-mediated signaling (657).

6. AT2 receptor function and signaling

The AT2 receptor is a seven-transmembrane domain protein consisting of 363 amino acids with a molecular mass of 41 kDa (604). AT2 and AT1 share 34% homology (604). AT2 receptors are highly expressed in various tissues of the developing embryo, but expression declines after birth (791, 792). The range of the AT2-dependent functions is still not completely understood. Similarly, the AT2-linked intracellular signaling events are still a matter of intense research and appear to be far more diverse than those of the AT1 receptor (for a comprehensive review, see Ref. 825). However, there is growing evidence that AT2-dependent signaling may antagonize the effects of ANG II on AT1 receptors under various conditions. This AT1-antagonizing effect of the AT2 receptor might be related, in part, to a direct interaction between the AT2 and AT1 receptors. The AT2 receptors were shown to form heterodimers with AT1 receptors, resulting in an attenuation of the AT1-mediated effects of ANG II (2). The AT2-mediated effects of ANG II on vascular resistance may serve as an example of how AT2 receptor activation functionally counteracts the AT1 receptor. Thus, in contrast to AT1 receptor-deficient mice, the AT2 knockout mice were shown to exhibit increased arterial blood pressure (328, 370). Commensurate with the vasodilator effect of AT2 activation, the infusion of ANG II under concomitant AT1 receptor inhibition resulted in a decrease in arterial blood pressure in rats, which was dependent on the intact NOS activity (116). Several other studies confirmed these findings and provided evidence that the ANG II binding to AT2 results in enhanced formation of vasodilator agents like NO, prostanooids, and bradykinin, as reviewed in detail elsewhere (951).

B. Renin/Pro-renin Receptor

Traditionally, the function of renin was thought to be restricted to its enzymatic activity, i.e., cleavage of the renin-substrate angiotensinogen. This assumption was first challenged by the finding that human plasma contains considerable amounts of the enzymatically inactive pro-renin (542). This phenomenon raised the question as to whether pro-renin in the circulation is somehow activated or transformed into active renin and/or whether pro-renin has a function that is entirely independent of the formation of enzymatically active renin, a function that might be related to the binding of pro-renin (and/or renin) to receptors.

The first evidence for the existence of a renin/pro-renin receptor was obtained by the finding that cultured human mesangial cells are able to bind renin, which subsequently induced the synthesis and release of the plasminogen activator inhibitor-1 (626). In a similar study, renin was shown to increase the expression of TGF-β1 and extracellular matrix proteins upon binding to mesangial cells, an effect that was independent of the renin activity (362). A few years after the discovery of the ability of renin to bind to mesangial cells, the human renin/pro-renin receptor was cloned (627). The renin/pro-renin receptor is a single-transmembrane 45-kDa protein consisting of 350 amino acids with no apparent homology to known receptors. This novel receptor can bind both pro-renin and renin (627). The binding of renin to its receptor resulted in a fivefold increase in the catalytic activity compared with renin in solution. The binding of pro-renin to the receptor increased its enzymatic activity from virtually zero to values comparable to those of active renin in solution (for details on renin/pro-renin receptor-independent activation of pro-renin, see sect. III A1). Although the exact mechanism of pro-renin activation upon receptor binding will require additional experimental efforts, it was suggested that pro-renin may employ its handle region to bind to the receptor, which would eventually expose the active site of the enzyme (627). Furthermore, the binding of renin to its receptor initiated the activation of the intracellular ERK1/ERK2 pathway independent of renin enzymatic activity (627). Thus the bind-
ing of renin and/or pro-renin to its receptor boosts the locally defined renin activity and is accompanied by intracellular signaling of the receptor-expressing cell. These findings subsequently sparked interest in the evaluation of possible clinical consequences of the renin/pro-renin receptor binding in vivo. To address this issue, a competitive inhibitor peptide was developed to prevent pre-renin/renin from binding to its receptor (755). The renin/pro-renin receptor inhibitor was used in rats during endotoxin-induced uveitis, since ANG II was shown to be crucially involved in the induction of ocular inflammation. The inflammatory parameters were markedly alleviated under the concomitant decoy peptide application (755). The therapeutic benefit of the renin/pro-renin receptor inhibitor appears to be substantial. The inhibition of renin/pro-renin receptor binding also markedly ameliorated the progression of cardiac fibrosis in stroke-prone SHR rats (368). In addition, the renin/pro-renin receptor inhibition was able to block MAPK activation and the development of glomerulosclerosis in a model of diabetic nephropathy (366). In a follow-up study in AT1a-deficient mice, the authors suggested an ANG II-independent effect that depended on pro-renin/renin receptor activation (416). In addition, ACE inhibition was shown to be substantially less efficient than the application of the pro-renin/renin receptor antagonist in preventing the development of diabetic nephropathy (416). With respect to the effect of the renin/pro-renin receptor inhibitor on the progression of cardiac fibrosis in SHR rats, the aforementioned results could not be confirmed without restrictions. Thus the prolonged infusion of the renin/pro-renin receptor antagonist was shown in another study to somewhat reduce the left ventricular mass in SHR rats, but it did not affect fibrotic cardiac remodeling, coronary hemodynamics, or left ventricular function (835). Similarly, the beneficial effect of pro-renin/renin receptor inhibition on renal function could not be confirmed in high-renin models of hypertension like the two-kidney one-clip model (543). In view of the outstanding relevance of the initial findings regarding the beneficial effects of pro-renin/renin receptor antagonism, future studies will be required to substantiate the initial reports and to confirm the results under modified experimental conditions.

In summary, a novel receptor type that binds renin and pro-renin with equal affinity was discovered. Upon receptor binding, a dual effect is observed. First, the specific enzymatic activity of both renin and pro-renin is substantially enhanced and, due to local renin/pro-renin trapping, restricted to the local environment of the receptor-expressing cells. Therefore, the renin/pro-renin binding may provide a basis for the locally restricted renin activity that might well exceed the magnitude of systemic renin activity. Second, the binding of renin/pro-renin appears to initiate intracellular signaling in the receptor-expressing cell, and this effect might be of relevance under pathophysiologic conditions.

C. The ANG-(1–7)-ACE2-Mas Axis

New components and functions of the renin-angiotensin system are still being discovered. Within the last decade, it has been recognized that the smaller peptide fragments of the RAS, including ANG III, ANG IV, and ANG-(1–7), also possess biological activity, but that their plasma values are much lower than those of ANG II, with the exception of ANG-(1–7) (321, 680). The identification of the ACE homolog ACE2, an important enzyme for the generation of ANG-(1–7), and of the G protein-coupled receptor Mas, which is encoded by the Mas protooncogene, as a receptor for ANG-(1–7), broadened our knowledge of the RAS towards a system containing at least two cascades, with the ACE2-ANG-(1–7)-Mas axis likely acting as a counterregulatory portion of the classical RAS axis. The increasing interest in this area of research has been documented by several reviews, in which the interested reader can obtain a more comprehensive overview (177, 223, 224, 424, 449, 498, 713, 752, 907).

1. Function of Ang-(1–7) and expression of ACE2 in the cardiovascular system

ANG-(1–7) can be formed from ANG I and ANG II by the action of ACE, ACE2, and several other enzymes, including neprilisin (NEP) and prolyl oligopeptidase (POP) (222), whereas ACE is the main enzyme responsible for the conversion of ANG I into ANG II. The carboxypeptidase ACE2 cleaves ANG II into ANG-(1–7) (912). Furthermore, ACE2 forms ANG-(1–9) from ANG I, which can then be converted into ANG-(1–7) by ACE (202). However, the preferable physiological substrate for ACE2 seems to be ANG II (878, 912). ANG-(1–7) is subsequently metabolized into the inactive fragment ANG-(1–5) by the activity of ACE (16, 139, 186). In addition, ACE2 is also capable of metabolizing other peptides, such as apelin-13, des-Arg9-[bradykinin], β-casomorphin-(1–7), dynorphin A-(1–13), ghrelin, and neurotensin-(1–11) (202, 912). The half-life of the heptapeptide ANG-(1–7) is very short (several seconds) in rats (962), and ACE inhibitors, which inhibit the metabolism of ANG-(1–7) into ANG-(1–5), clearly increase the half-life of ANG-(1–7) (962). The 805-amino acid enzyme ACE2 is a monocalboxypeptidase with one active site domain that shares ~42% homology with the active site of ACE (202, 878, 912). ACE2 gene expression has been found in the heart, kidney, and testes and, to a lower extent, in the liver, lung, small intestine, and brain of humans and rodents (259, 303, 451). ACE2 is an ectoenzyme, although a soluble active form has also been described in plasma and urine (790, 939).
The first biological activities of ANG-(1–7) to be described were the demonstration of AVP release from the hypothalamo-neurohypophyseal system and the decrease of arterial blood pressure after microinjection of low doses of ANG-(1–7) into the dorsal medulla of rats (111, 757). Further studies have demonstrated immunostaining of ANG-(1–7) in central regions related to the synthesis and release of vasopressin (79, 110, 467). Subsequent studies have shown that ANG-(1–7) is involved in the baroreflex control of the heart rate at the nucleus tractus solitarii (67, 112, 143, 645, 742), enhances the long-term potentiation in the hippocampus (330), and contributes to the maintenance of sympathetic nerve activity in the neurons of the paraventricular hypothalamic nucleus (805). In line with these observations, ACE2 expression has been reported in various cardiovascular regulatory brain areas (203, 742). ACE2 protein expression was found to be lower in the rostral ventrolateral medulla of SHR compared with normotensive Wistar-Kyoto rats (WKY), and vascular or central overexpression of ACE2 was found to decrease blood pressure in SHR (724, 965).

With regard to the heart, it has been found that ANG-(1–7) evokes antiarrhythmic effects (226, 751), dilates the coronary arteries (87), increases coronary blood flow (123, 124), and affects the cardiac contractility (225, 750). The cardiac expression of ACE2 has been localized to endothelial cells and smooth muscle cells from the intramyocardial vessels and myocytes (104, 202, 878). ACE2-deficient mice show reduced cardiac contractility, a decrease in aortic and ventricular pressure, and a thinning of the left ventricular wall. These changes progressed with age and were more prominent in male mice. The hearts of ACE2 knockout mice exhibited enhanced ANG II levels and increased levels of hypoxia-inducible genes (170). However, these effects seem to be dependent on the background of the ACE2-deficient mice (288). Increased cardiac ACE2 expression and activity have been found in failing human and rat hearts following arterial injury and ACE2 gene expression has been described for gliovascular smooth muscle cells, where ANG-(1–7) can induce the release of NO via eNOS (744). In addition to its effect on NO release, ANG-(1–7) stimulates the release of prostanoids from endothelial and vascular smooth muscle cells (393, 394, 613). Moreover, ANG-(1–7) seems to potentiate the vasodilatory effect of bradykinin (87, 519, 675, 676) and attenuate the vasoconstrictor effect of ANG II (805). However, vasoconstriction in response to ANG-(1–7) has also been reported (1, 656). In addition, ANG-(1–7) also acts on vascular smooth muscle cells, where it inhibits cell growth (233, 846). More recently, ANG-(1–7) has been proposed to attenuate atherosclerotic plaques in animals (201, 540).

The pulmonary ACE2 expression has been described for type I and type II alveolar epithelial cells, bronchiolar epithelial cells, endothelial cells, and arterial smooth muscle cells (303, 372, 471). ACE2 expression decreases with aging in rat lungs and is higher in old female rats compared with old male rats. The ACE2-deficient mice do not show any pulmonary abnormalities (170). However, ACE2 deletion worsens the severe acute lung injury induced by acid aspiration or sepsis, and the injection of recombinant human ACE2 attenuates the acute lung failure in the ACE2 knockout as well as wild-type mice (372). In addition, ACE2 is a well-described receptor for the severe acute respiratory syndrome coronavirus (SARS-CoV) and for the human coronavirus-NL63 (348, 515). The SARS-CoV infection can be attenuated by soluble ACE2 molecules, ACE2 antibodies, and the genetic deletion of ACE2 (304, 472, 515).

Several studies have demonstrated that the kidney is an additional target for the action of ANG-(1–7). ANG-(1–7) injection has been shown to increase the sodium and water excretion, as well as the GFR, without affecting the renovascular resistance in isolated perfused rat kidneys and in anesthetized animals (189, 899). Subsequent studies have found that ANG-(1–7) modulates the activity of Na⁺-K⁺-ATPase in the basolateral membranes of kidney proximal tubules (121, 305). In addition, ANG-(1–7) has been shown to stimulate the arachidonic acid release from isolated proximal tubules (26), and the inhibition of prostaglandin release by COX inhibition attenuates the ANG-(1–7)-induced increase in urine flow and sodium excretion (336). ANG-(1–7) has also been shown to cause afferent arteriolar vasodilation and to antagonize the vasoconstrictor effects of ANG II (32). The intrarenal blockade of ANG-(1–7) causes a decrease in GFR, renal plasma flow, and sodium excretion in rats with increased activity of renal RAS (100). However, ANG-(1–7) also induces antiinflammation in water-loaded rodents (748) and increases renal tubular sodium reabsorption in rats (101).

ACE2 gene expression has been described for glomeruli, vasa recta, and all nephron segments except the mTAL (514). Relatively high amounts of ACE2 were detected in the apical brush-border membrane of proximal tubule epithelia, where it colocalized with ACE (514).
Within the glomerulus, ACE2 is localized in podocytes and, to a smaller extent, in the mesangial cells (974). The increased renal ANG-(1–7) levels and renal expression of ACE2 have been found during pregnancy (88, 410) and in response to ACE inhibition or ANG-II-AT1 receptor antagonism (404, 876). Renal ACE2 expression was found to be lower in adult SHR compared with age-matched WKY rats during lipopolysaccharide-induced acute renal failure and in several models of diabetic kidney injury (287, 875, 876, 974, 988). Several findings have suggested a protective role for the ACE2-ANG-(1–7)-Mas axis during diabetic nephropathy: 1) ACE2 gene deletion and pharmacological ACE2 inhibition with the ACE2 inhibitor MLN-4760 worsen the development of glomerular injury in diabetic mice (819, 957), 2) treatment with ANG-(1–7) or the Mas receptor agonist AVE0991 ameliorates diabetes-induced renal and cardiovascular dysfunction (69, 70, 210), 3) attenuation of diabetic renal injury by ACE inhibition is paralleled by the increased ACE2 expression (876), and 4) increased ACE2 expression in streptozotocin-induced diabetic mice has been reported (960). However, ACE2 polymorphisms and diabetic nephropathy do not seem to be associated in humans (242). A recent study reported that loss of the ACE2 gene leads to the development of ANG II-dependent glomerular injury in male mice, but not in female mice. Moreover, these authors found that the greater degree of glomerulosclerosis and proteinuria was attenuated by ANG II-AT1 receptor inhibition (661). In humans, the de novo expression of ACE2 in biopsies of patients with renal diseases and an increased ACE to ACE2 ratio in subjects with hypertension have been reported (508, 930).

2. Mas receptor

The existence of a receptor for ANG-(1–7) was initially suggested by pharmacological studies using ANG-(1–7) antagonists (848). The orphan heterotrimeric guanine nucleotide-binding protein-coupled receptor, i.e., the Mas receptor, was later suggested to be the functional binding partner for ANG-(1–7) (510). The Mas gene encodes for a G protein-coupled receptor and was originally detected in vivo by tumorigenic properties originating from the rearrangement of its 5’ region flanking a protooncogene, due to its tumorigenicity (708, 979). The genetic deletion of the Mas receptor has been shown to eliminate the binding of ANG-(1–7) to mouse renal cells, leading to impaired cardiac and renal function and to attenuation of the vasodilator effects of ANG-(1–7) (753) (Fig. 7). The Mas gene is expressed in the brain, testes, kidney, and heart (391, 581, 978). Within the central nervous system, the Mas receptor expression has been found in various regions, including the cardiovascular regulatory areas (58). In agreement with the activities described for ANG-(1–7), Mas-deficient mice exhibit increased blood pressure, impaired endothelial function, decreased NO production, and decreased endothelial NO synthase expression (679, 961). In line with the cardioprotective effects of ANG-(1–7), the genetic deletion of the Mas receptor impairs heart function and changes the extracellular matrix to a profibrotic state (750). In addition, the genetic deletion of the Mas receptor leads to changes in glucose and lipid metabolism (754), erectile function (176), heart function (123, 750), and brain function (330). The intracellular signal transduction mechanisms that follow the activation of the Mas receptor are only poorly understood. ANG-(1–7) stimulates the phosphorylation of Janus kinase 2 (JAK2) and insulin receptor substrate (IRS)-1 in the rat heart in vivo (266). Mas receptor activation leads to an increase in the NO production via the phosphorylation of eNOS, which involves the activation of the phosphatidylinositol 3-kinase-dependent Akt phosphorylation (192, 744). Furthermore, the inhibition of MAPK phosphorylation upon activation of the Mas receptor has been described (830, 847). In addition, the Mas deficiency impairs Ca2+ management in cardiomyocytes (192). In the Mas-transfected CHO and COS cells, ANG-(1–7) stimulated the release of arachidonic acid (753).

It should be noted, however, that some studies have shown that ANG-(1–7) can also bind to the AT1 and AT2 receptors, although rather high concentrations of ANG-(1–7) are necessary (552, 730, 931). In addition, the exis-
tence of another ANG-(1–7) receptor has been suggested (806), and an interaction between the different angiotensin receptors has been proposed (124, 460).

VII. CONCLUSION

Despite the long-standing awareness of renin production and secretion, our knowledge of its regulation and (patho)physiological relevance has steadily increased during the last few years. We have begun to understand the fundamental properties of renin gene regulation: in particular, its stimulation by the cAMP pathway and (mostly) inhibitory modulation by specific hormones and cytokines. We have improved our knowledge of the development, cell biology, and function of renin-producing cells in the kidney, commonly referred to as JG cells. The discovery of the functional relevance of gap junctions in JG cells has revealed novel and promising insights into the cell biology of renin-producing cells. Although the cAMP signaling pathway is known as a very powerful stimulator of renin secretion, the detailed mechanisms of renin release are still obscure and await further clarification. The mysterious “calcium paradox,” referring to the inhibitory effect of calcium on secretion in renin-producing cells, may have found at least a partial explanation in the existence of calcium-inhibitable adenylyl cyclases in JG cells. ATP, NO, and prostanooids have emerged as powerful signals that mediate the local control of renin secretion and synthesis. Furthermore, the list of hormones that act directly on JG cells has been substantially enlarged. ANG II, the classic biological effector of the RAS, has been shown to act through different receptors that mediate antagonistic effects. Moreover, ANG II is probably not the only biological mediator of the renin-angiotensin system because (pro)renin and ANG-(1–7) were also found to exert direct and distinct biological effects. The knowledge about kidney renin is certainly not yet complete, although 100 years have passed since its discovery. We may therefore anticipate with interest the novel findings about kidney renin that are expected in the upcoming years.

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