[Frontiers in Bioscience, Scholar, 5, 198-205, January 1, 2013]

Cytosolic (pro)renin and the matter of intracellular renin actions

Jorg Peters[1]

[1]*Institute of Physiology, University of Greifswald, Greifswalder Str. 11C, D-17495 Karlsburg, Germany*

TABLE OF CONTENTS

1. ABSTRACT

Renin is known as a secretory glycoprotein produced, stored and released by the kidney. Renin cleaves angiotensin (ANG) I from angiotensinogen, which is further cleaved to ANG II by ANG-converting enzyme. ANG II acts via specific receptors located at the cell membrane. Recently renin transcripts have been discovered which encode a cytosolic protein that cannot be secreted. These transcripts are derived from the same renin gene but probably use another promoter located within intron A. Whereas the kidney exclusively expresses the transcript encoding secretory renin, other tissues additionally or exclusively express transcripts encoding cytosolic renin. The cytosolic renin protein exerts functions different and even opposite to those of secretory renin. Whereas secretory renin increases necrotic death rates of cardiac cells, the cytosolic renin isoform even protects cells from necrotic death. This review describes the properties of cytosolic renin and its cellular functions and discusses possible mechanisms of actions particularly in the light of the discovery of direct nuclear and mitochondrial effects of ANG' s.

2. INTRODUCTION

The circulating renin-angiotensin system (RAS) modulates blood pressure, water balance, electrolyte homeostasis and inflammatory as well as growth processes. The activity of the system is determined predominantly by renin. Renin cleaves angiotensin (ANG) I from its only known substrate, angiotensinogen. ANG I is then cleaved by ANG I-converting enzyme to ANG II, the effector peptide of the system. Renin also binds to a recently discovered (pro)renin receptor (1) thereby exerting ANG-dependent as well as ANG-independent effects [for review see (2)]. Renin is commonly known as a secretory protein produced by the kidney. Here, renin is stored within lysosome-like granules (3) and secreted in a regulated manner. Production of ANG by renin can occur extracellularly or within secretory vesicles (4). Surprisingly, renin immunoreactivity (IR) was also found within mitochondria (5, 6), which is quite an unusual place for a secretory protein to be. Nevertheless, this observation appeared to be more than an artifact, since not only renin IR but also renin enzymatic activity was detected within mitochondrial fractions (6). Furthermore, it was shown that

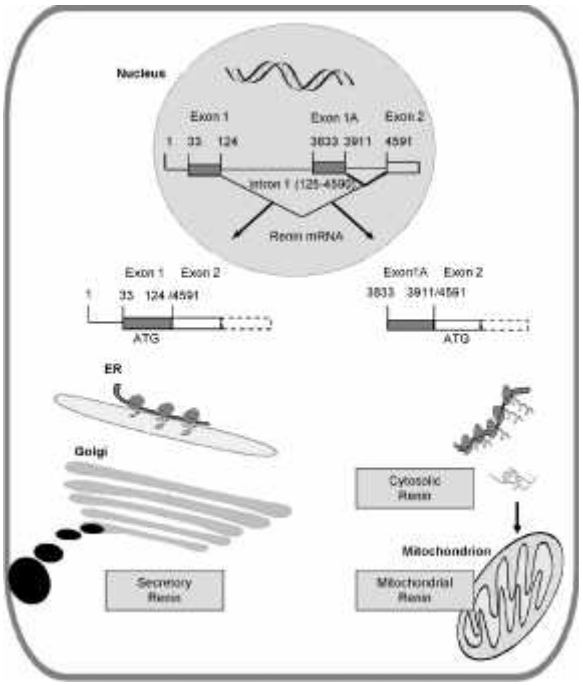

Figure 1. Renin Transcripts derived from the rat renin gene encode for either secretory or cytosolic renin. A: Co-translational transport of exon(1-9)renin to the rough endoplasmatic reticulum and modification at the Golgi apparatus results in renin proteins that are membrane bound and can be secreted. Secretory renin generates angiotensin I from angiotensinogen at extracellular sites and also binds and activates the (pro)renin receptor, thereby exerting angiotensin-dependent and angiotensin-independent effects. B: Translation of exon (2-9)renin at free ribosomes results in cytosolic renin proteins that can be imported into mitochondria, but cannot be secreted. These proteins act at intracellular sites and can protect cells from necrotic cell death. The mechanisms of action of cytosolic renin are presently unknown.

isolated mitochondria were able to specifically import prorenin (7). Thus, it is obvious to pursue the hypothesis that a non-secretory renin exists, which exerts its own functions different from those of the circulating renin-angiotensin system. The role of non-secretory renin will be discussed particularly in the light of the recently reported angiotensin effects in the cytosol, nuclei and mitochondria (see below).

3. PREVIOUSLY UNRECOGNIZED RENIN TRANSCRIPTS ENCODE A CYTOSOLIC RENIN ISOFORM

Secretory proteins are targeted by means of a co-translational transport to the endoplasmatic reticulum (ER) where translation will be completed and the proteins are packed into vesicles. This transport requires an ER translocation signal commonly present at the N-terminus of the protein. Any protein that contains such a signal will be unvariably transported to the ER. In contrast, isoforms of renin that are to be imported into mitochondria - according to common belief - must be produced at free ribosomes in the cytosol and should therefore better not contain the ER translocation signal. The translocation signal for renin to the ER is encoded by a sequence derived from exon 1 of the renin gene. Using a 5'-RACE protocol, we and others have isolated and characterized a renin transcript termed exon(1A-9)renin for the rat (7) or renin-b (8, 9). The exon(1A-9)renin transcript lacks exon 1 and thus indeed the signal peptide for the transport to the ER, whereas and the other exons (2-9 in the rat) are the same. The transcript is derived from the same renin gene as the transcript for secretory renin. In contrast to the latter, exon 2 of the exon(1A-9)renin transcript is preceded by a short sequence of about 80 base pairs, termed exon1A, which is derived from intron A and not from exon 1 (7). As it turned out, exon1A does not contain any in-frame ATG and thus represents a 5' untranslated region (UTR) rather than a true coding exon. This 5' UTR may have as yet unknown regulatory functions which are presently under investigation.

The translation start for the non-secretory renin isoform resides within the first ATG in exon 2. The transcript encodes for a truncated prorenin that lacks the prefragment with the ER translocation signal as well as 15 amino acids of the prosegment of secretory prorenin. Using canine microsomal membranes, Lee-Kirsch *et al.* demonstrated *in vitro* that this truncated prorenin – in contrast to the secretory exon(1-9)renin transcript-derived prorenin - indeed cannot be processed into the secretory pathway by the ER (8). In addition to the prefragment, the prosegment of prorenin also appears to be important for correct secretion. Deletion of the complete prosegment of secretory preprorenin, even when leaving the prefragment intact, resulted in a 99% decrease of the renin secretion rate, when compared with the native protein (10). Moreover, already a single amino acid exchange can inhibit the secretion of human renin markedly (10). The inhibition of secretion may be a result of folding, targeting or diminished stability. Given that the prosegment or single amino acids of it affect the secretion rate it has to be asked whether or not (and to which degree) the rat cytosolic truncated prorenin molecules is folded "correctly" with respect to the renin protein we have become acquainted so far. The cytosolic renin lacks 15 N-terminal amino acids (LPTDTASFGRILLKK).

Nevertheless, there is evidence that at least a considerable portion of the cytosolic truncated prorenin is folded well to such an extent that it yields an enzymatically active protein, capable of cleaving angiotensinogen. This was shown using a reticulocyte-lysate *in vitro* translation system (7) or overexpression of cytosolic truncated prorenin in ATT20 cells (8) and in H9c2 cells (11). Still indeed we observed that the amount of renin with enzymatic properties produced by overexpression of cytosolic truncated prorenin was much lower than the amount of renin produced by a similar degree of overexpression of secretory renin.

As deduced from the absence of the prefragment truncated cytosolic prorenin remains intracellularly (Figure 1). In agreement with this concept, cells that express

cytosolic renin failed to secrete renin into the medium (8, 11) and overexpression of rat cytosolic renin in transgenic rats failed to cause elevated circulating renin or prorenin levels whereas it markedly increased tissue renin activities (12). Furthermore, increased transcript levels of human cytosolic renin in the brain of a transgenic mouse model did not result in measurable levels of renin in the cerebrospinal fluid (13).

We observed renin IR in H9c2 cells, particularly within mitochondria, by means of confocal microscopy and an enrichment of renin enzymatic activity within the mitochondrial fractions (11). In these cells, overexpression of the truncated prorenin resulted in an increase of renin concentrations in the mitochondrial fractions but not in the medium, whereas overexpression of secretory renin resulted in increased concentrations of renin in the lysosomal fractions as well as in the medium but not specifically within mitochondria (Figure 1).

In transgenic rats overexpressing the truncated prorenin, renin enzymatic activity was found particularly in the cytosol and in mitochondrial fractions of the heart and adrenal gland, indicating the existence of a cytosolic and mitochondrial non-secretory renin isoform. These data support initial observations of renin IR and renin enzymatic activity in mitochondria of the rat adrenal cortex (6). In support of a mitochondrial localization of renin, we demonstrated that the truncated prorenin, but not the secretory preprorenin nor active renin can be imported into isolated adrenal mitochondria *in vitro* (7). This mitochondrial import was shown to be an active process, since it dependents on an intact mitochondrial electron transport and intact oxidative phosphorylation. Correct folding of the renin protein may here not be necessary, since most mitochondrial proteins are imported in a more or less unfolded state (14).

4. DIFFERENTIAL REGULATION AND TISSUE-SPECIFIC EXPRESSION OF RENIN TRANSCRIPTS

Transcripts encoding a cytosolic, non-secretory isoform of prorenin have been found in rats, mice and humans (7, 9). One of these transcripts, termed renin-b, was assumed to be exclusively expressed in the brain (8, 9). Another transcript termed renin-c, exhibiting a 79 bases extension at the 5' end of exon 2, was described to be expressed specifically in the lung (9). In our hands, most but not all tissues of the rat express both, the exon(1-9)renin transcript encoding for secretory renin and the exon(1A-9)renin transcript encoding for cytosolic renin. The latter corresponds to the renin-b transcript, except that in renin-b only 12 base pairs of exon1b are derived from intron A of the rat renin gene whereas we detected a segment of 80 base pairs in exon1A derived from intron A. The rat kidney exclusively expresses the exon(1-9)renin transcript encoding secretory renin whereas the rat heart expresses exclusively the exon(1A-9)renin transcript encoding cytosolic renin (15). Interestingly, in the heart, transcript levels for cytosolic renin (but not for secretory renin) increase considerably after myocardial infarction, indicating that cytosolic renin may play a role particularly under ischemic conditions.

5. FUNCTIONS OF CYTOSOLIC (PRO)RENIN

Since in the rat heart the transcript encoding cytosolic renin is upregulated under ischemic conditions it was of interest to investigate the effects of overexpression of this transcript in cardiac cells. To determine whether or not cytosolic renin exerts functions that are different from those elicited by secretory renin, we compared the cellular responses to the overexpression of either transcript in rat H9c2 cardiomyoblasts. These cells were particularly valuable for our questions, because, Like the rat heart, H9c2 cardiomyoblasts do not express secretory renin and basal expression of cytosolic renin is weak rendering these cells well suited to investigate the current issue. Overexpression of secretory renin in H9c2 cells resulted in a hypertrophic response, which is in agreement with the known effects of the circulating renin-angiotensin system. However, with increasing duration of culture time, cells expressing secretory renin exhibited a marked increase in necrotic deaths as indicated by the amount of lactate dehydrogenase (LDH) released normalized to LDH content (11). In contrast, overexpression of cytosolic renin inhibited the increase in necrosis rate that normally occurs when cells are stressed (due to an increase in cell concentrations over the culture time): In control transfected H9c2 cells the rate of necrosis increased from about 20% per day (days 1-6) to about 30% and 50% at days 8 and 9, respectively. In cells overexpressing secretory renin, the rate of necrosis increased from 20% within the first six days of culture to as high as about 80% and 95% at days 8 and 9, respectively ($p<0.05$). In contrast, in cells overexpressing cytosolic renin the rate of necrosis did not increase at all and remained low at about 20% for up to ten days of culture ($p<0.05$ vs. controls and vs. cells overexpressing secretory renin). From these data, we conclude that cytosolic renin is functionally active and exerts independent effects which are different from and even opposite to those of circulating renin.

Whereas it is well known that the circulating renin-angiotensin system can be deleterious to cardiac cells, cytosolic renin appears to be rather cardioprotective. The increased cardiac expression of cytosolic renin after myocardial infarction (15) may represent a beneficial mechanism targeted against ischemic stress or a mechanism that promotes healing. On the other hand, cells overexpressing cytosolic renin show an increased initial rate of apoptosis as compared to control transfected cells or cells overexpressing secretory renin (11).

Transgenic rats overexpressing cytosolic renin driven by an exon(2-9)renin construct under control of the CXCMV promoter appear to be perfectly healthy with normal blood pressure levels and low levels of circulating renin (12). This observation supports the notion that cytosolic renin dos not contribute to the activity of the circulating renin-angiotensin system. Interestingly, the cytosolic and intra-mitochondrial renin levels were increased in the hearts and adrenal glands of exon(1-9)renin transgenic rats. Concomitantly, the plasma aldosterone-to-renin-ratio was elevated, albeit without a detectable

increase in absolute aldosterone concentrations. The increased aldosterone-to-renin ratio indicates that plasma renin-independent mechanisms of aldosterone production are stimulated in these transgenic rats. The mechanism in question could well be related to cytosolic or mitochondrial renin, although this issue remains to be investigated. Furthermore, since blood samples for measurements of aldosterone and renin were taken from rats under ether anesthesia the data need to be confirmed with blood samples taken from conscious rats.

Lavoie *et al.* generated transgenic mice in which the transcript for either human secretory renin or for human cytosolic renin was specifically overexpressed in brain astrocytes (13). Unfortunately, mouse angiotensinogen is a poor substrate for human renin. To investigate the enzymatic activity of human renin and the functional consequences of this activity *in vivo* in these rats, it was therefore necessary to generate double transgenic mice overexpressing both, human renin and human angiotensinogen. Interestingly in both double transgenic mice lines (angiotensinogen plus secretory renin and angiotensinogen plus cytosolic renin) blood pressure and water intake were increased, suggesting that not only secretory renin but also cytosolic renin expressed in astrocytes may contribute to the regulation of blood pressure and water balance. Unfortunately, this conclusion is somewhat hampered by the fact that the increase in renin activity seen in single transgenic mice overexpressing the transcript encoding cytosolic renin was not strictly limited to brain astrocytes but was also found in the plasma, although to a minor extend. It also remains to be demonstrated that the overexpression of the transcript encoding cytosolic renin stimulates the production (translation, correct folding etc.) of cytosolic renin protein in brain astrocytes in this model. The same group elegantly demonstrated that the preservation of intracellular renin cannot compensate for the defects induced by the loss of secretory renin (16): Using a cre-lox strategy, Xu *et al.* were able to specifically delete exon 1 from the renin gene, resulting in the complete absence of secretory renin whereas cytosolic renin was preserved (16). The transgenic mice exhibited (besides others) low arterial pressure and were unable to produce a concentrated urine. Furthermore, they exhibited a poor survival at weaning and develop renal lesions. This phenotype is very similar to the phenotypes induced by the complete deletion of either renin, angiotensinogen, or angiotensin receptors type 1 (17-20). Interestingly, in angiotensinogen-deficient mice the brain-specific restoration of ANG II through a vector directing the ANG peptide to the secretory pathway is able to prevent renal defects (21). Taken together, these data suggest that ANG production through local secretory renin in the brain is essential for renal development and that cytosolic renin cannot compensate for the loss of the secretory system (16).

6. MECHANISM OF ACTION OF INTRACELLULAR RENIN

6.1. ANG II and its effects in cytosol, mitochondria and nuclei

Given that the overexpression of cytosolic renin has functional consequences which are independent from the action of secretory renin, the question arises as to the potential mechanisms of action of cytosolic or mitochondrial renin. What are the targets of cytosolic or mitochondrial renin? And how are the effects accomplished? One hypothesis readily at hand is that cytosolic renin, like secretory renin, generates ANG I from angiotensinogen, the so far only known substrate of renin, within the cytosol, mitochondria or nuclei. Then angiotensins (ANG II, ANG III, ANG IV etc.) may mediate the effects of renin via angiotensin receptors at these sites. There are several arguments in favor of this hypothesis but some open questions remain:

6.1.1. Cytosolic, intra-mitochondrial or nuclear angiotensin generation: supporting data

1) There is evidence for the presence of renin, ANG I-converting enzyme (ACE), ANG's, and ANG II receptors (ATRs) in the cytosol (22), the nuclei (22-24) and the mitochondria (5, 6) of extrarenal tissues, such as liver and adrenal gland., These observations gave rise to the speculation that there may be an independent intracellular renin-angiotensin system that may have functions which different from those of the circulating system.

2) In isolated heart muscle cell pairs the intracellular dialysis of renin decreased the junctional conductance between the cells and this effect was potentiated by concomitantly dialysed angiotensinogen. The effect of renin was blocked by dialysis of the ACE inhibitor enalaprilat (25).

3) In primary vascular smooth muscle cells ANG II injected into the cytoplasm increased intracellular free calcium levels in the cytosol and in the nucleus. These effects were blocked by the concomitant injection of the AT1R blocker CV-11947 even after desensitization of cell membrane-bound ANG receptors (26). ANG II was here identified in endosomes as well as in the nucleus.

4) In isolated cardiomyocytes dialysis of ANG II increased inward calcium currents and this effect was blocked by inhibitors of protein kinase C (27). Another group observed an increase of calcium currents induced by dialysed ANG II in cardiac myocytes and demonstrated that this effect was mediated by the AT1R (28).

5) When rat hepatoma cells were transfected with a vector designed to overexpress a non-secretory angiotensinogen in the cytosol they exhibited an increased mitogenic index that was blocked by losartan or by a renin antisense phosphorothioate oligomer (29).

6) Co-expression of ANG II fused to cyan fluorescent protein together with AT1R receptor fused to yellow fluorescent protein resulted in an enrichment of both proteins in the nucleus and enhanced cell proliferation as well as an enhanced activity of the cAMP response element-binding protein (CREB) in COS-7 and CHO-K1 cells, respectively (30). It is currently unknown whether these nuclear effects are direct or indirect effects of ANG II.

7) In isolated nuclei from rat cardiomyocytes ANG II stimulated the de novo synthesis of RNA in general

and of NFkappaB RNA in particular (31). It was demonstrated that both AT1R and AT2R, were located in the nuclear membranes and that the ANG II-induced effects on RNA synthesis were medidated by these receptors. Furthermore, it was shown that the effects of ANG II on isolated nuclei depended on the presence of pertussis toxin-sensitive Gi proteins. The AT1R-mediated effects were further shown to involve calcium release from calcium stores of the nuclear envelope by an IP3-dependent pathway. These data indicate that classical G protein-coupled receptor signal transduction takes place in the nuclei.

8) Also in mitochondria a local renin-angiotensin system has been reported. Abadir *et al.* (32) identified and characterized a complete functionally active mitochondrial renin-angiotensin system in human skeletal muscle cells, monocytes, mouse cardiac myocytes, renal tubular cells, neuronal cells, vascular endothelial cells and hepatocytes. In this study (32) functional AT2Rs in the inner mitochondrial membrane were identified by means of high-resolution transmission immunoelectron microscopy. The receptors were colocalized with ANG II. Immunoreactivity for AT_2R and ANG II was found in both whole cells and in isolated mitochondria. Furthermore, a GFP (green fluorescence protein)-AT2R fusion protein colocalized with the mitochondrial marker MitoTracker Red. Finally, the effects of AT2R agonists and antagonists on NO production and mitochondrial respiration were investigated in isolated mitochondria. The AT2R agonist CGP421140 caused a dose-dependent increase in mitochondrial NO production and a decrease in mitochondrial respiration. These effects were partially blocked by the AT2R antagonist PD-123319. This list is not intended to be comprehensive but rather to convey an idea of how dynamic this field currently is. Taken together, there is ample evidence to suggest that complete renin-angiotensin systems may exist within single cells. On the other hand, for many of the studies described above the task still remains to validate the specificity of the functional observations using appropriate ATR knock out or knock down models, which would be the most informative controls. In other words, for the final acceptance of the concept that a functionally relevant intracellular renin-angiotensin system exists, it needs to be shown that the deletion of one or both ATR abolishes the cellular effects of intracytoplasmatically, intranuclearly or intramitochondrially applied ANG II, ATR agonists or ATR antagonists.

6.1.2. Cytosolic, intra-mitochondrial or nuclear angiotensin generation: open questions

Although the data presented above support the hypothesis of cytosolic or mitochondrial ANG generation and function, there are still some open questions: To generate ANG in the cytosol or within mitochondria the cytosolic or mitochondrial renin must act on and thus be in contact with angiotensinogen. Angiotensinogen, however, is a secretory protein. There is no evidence to date that angiotensinogen can be produced in the cytosol or that it can reach the cytosol from intracellular vesicles of from the extracellular space where the protein is normally found. So far no transcript has been found that may encode a cytosolic angiotensinogen and it is unlikely that such a transcript will be found. The same holds true for the other components of the renin-angiotensin system, ACE and ATR. The binding sites (for ANG I or ANG II) are either directed to the extracellular space or to the inside of vesicles, but not to the cytosol. Although we did not detect angiotensinogen within mitochondria we could demonstrate ACE and renin immunoreactivity in mitochondria of the rat adrenal cortex (6). Nevertheless, IR is only a weak indicator and prone to produce artifacts, as long as it is not validated using the respective knock out models. We may still need to wait for the discovery of new transcripts that allow the cytosolic synthesis and/or mitochondrial import of these components. Therefore, the presence of ANG's in the nucleus, the mitochondria or the cytoplasm is probably not the result of local production and it remains elusive how the peptides get to these sites. If they were taken up from the extracellular space, ANG's should be packed within vesicles as a result of receptor-mediated endocytosis. Also, it is currently unkown how ANG's may exit their vesicles to reach the cytoplasm and/or the mitochondria. Recently, a concept of "leaky endosomes" has been proposed that may provide a potential mechanism for intravesicular ANG's to reach the cytoplasm (33). Another possibility could be the existence of specific "channels" or peptide transporters that may translocate ANG from the extracellular space or from granules into the cytosol, but again, such systems have not yet been found.

6.2. ANG II-independent effects of renin in cytosol, mitochondria and nuclei

Besides angiotensinogen two other binding partners for renin have been discovered, namely the (pro)renin receptor (1) and the renin-binding protein, RnBP (34). The (pro)renin receptor mediates ANG-dependent and ANG-independent effects. Like angiotensinogen, the (pro)renin receptor can well bind secretory renin, but is unlikely to meet cytosolic renin: the binding site for (pro)renin within the (pro)renin receptor is directed to the extracellular space or to the lumen of vesicles, but not to the cytosol. The RnBP, on the other hand, may well meet cytosolic, but not secretory renin. In agreement with observations presented above, knock out of the RnBP had no effect on the cardiovascular systems (e. g. normal blood pressure as well as water and electrolyte balances) (35). The RnBP inhibits renin activity when binding renin and also has epimerase activity. We presently investigate the interaction between these partners in detail.

In addition to its ANG II-dependent effects, mitochondrial renin may have so far unknown direct targets within mitochondria. In this context the observation of an interaction between a particular renin gene allele (REN 10) and the mitochondrial H haplotype that favors longevity may be of interest (36). Mitochondria are the site of aldosterone and ATP production and contribute to the initiation of apoptosis. Overexpression of cytosolic renin increased the aldosterone-to-renin ratio in transgenic rats (12) and modulated particularly mitochondrially initiated apoptosis (11). These effects may be direct on the mitochondrial level; however, so far no mitochondrial renin-binding partner has been reported.

7. CONCLUSIONS

There is no doubt that renin-angiotensin systems exist locally in various tissues. Some of them act like the secretory system and may modulate or amplify the effects of the circulating system, for example in the adrenal gland (37-40). Here, renin may be expressed locally or taken up from the circulation ([or review see: (37, 40)]. In addition, evidence now accumulates that renin and angiotensin can act directly within cells at the cytosolic, mitochondrial or nuclear levels. Some of these effects are even opposite to those elicited by the secretory system. Whereas secretory renin may damage cells in the cardiovascular system, leading to necrotic cell death and/or fibrosis the cytosolic renin variant protects cells from necrotic death. Whether or not and to which degree this may be of importance for survival or regeneration, for example after myocardial infarction where expression of cytosolic renin increases, remains to be shown. The mechanism(s) of action of cytosolic renin and the source(s) for cytosolic angiotensin are currently unknown and await further clarification.

8. ACKNOWLEDGEMENTS

Part of the work was supported by the "German Research Foundation (DFG)" (grants PE 366/3 and PE 366/11), the "German Federal Ministry for Education and Research" (NBL3 program, reference 01 ZZ 0403) and the "German Foundation for Heart Research" (Deutsche Stiftung für Herzforschung; F/07/04).

9. REFERENCES

1. Nguyen G., F. Delarue, C. Burckle, L. Bouzhir, T. Giller and J. D. Sraer: Pivotal role of the renin/prorenin receptor in angiotensin II production and cellular responses to renin. *J Clin Invest* 109(11), 1417-27 (2002)

2. Nguyen G. and A. H. Danser: Prorenin and (pro)renin receptor: a review of available data from *in vitro* studies and experimental models in rodents. *Exp Physiol* 93(5), 557-63 (2008)

3. Hackenthal E., M. Paul, D. Ganten and R. Taugner: Morphology, physiology, and molecular biology of renin secretion. *Physiol Rev* 70(4), 1067-116 (1990)

4. Inagami T., M. Nakamaru, K. N. Pandey, M. Naruse, K. Naruse, K. Misono, T. Okamura and M. Kawamura: Intracellular action of renin, angiotensin production and release. *J Hypertens Suppl*, 4(4), S11-6 (1986)

5. Rong P., J. L. Berka, D. J. Kelly, D. Alcorn and S. L. Skinner: Renin processing and secretion in adrenal and retina of transgenic (mREN-2)27 rats. *Kidney Int* 46(6), 1583-7 (1994)

6. Peters J., B. Kranzlin, S. Schaeffer, J. Zimmer, S. Resch, S. Bachmann, N. Gretz and E. Hackenthal: Presence of renin within intramitochondrial dense bodies of the rat adrenal cortex. *Am J Physiol* 271(3 Pt 1), E439-50 (1996)

7. Clausmeyer S., R. Sturzebecher and J. Peters: An alternative transcript of the rat renin gene can result in a truncated prorenin that is transported into adrenal mitochondria. *Circ Res* 84(3), 337-44 (1999)

8. Lee-Kirsch M. A., F. Gaudet, M. C. Cardoso and K. Lindpaintner: Distinct renin isoforms generated by tissue-specific transcription initiation and alternative splicing. *Circ Res* 84(2), 240-6 (1999)

9. Sinn P. L. and C. D. Sigmund: Identification of three human renin mRNA isoforms from alternative tissue-specific transcriptional initiation. *Physiol Genomics* 3(1), 25-31 (2000)

10. Mercure C., G. Thibault, S. Lussier-Cacan, J. Davignon, E. L. Schiffrin and T. L. Reudelhuber: Molecular analysis of human prorenin prosegment variants *in vitro* and *in vivo*. *J Biol Chem* 270(27), 16355-9 (1995)

11. Wanka H., N. Keßler, J. Ellmer, N. Endlich, B. S. Peters, S. Clausmeyer and J. Peters: Cytosolic renin is targeted to mitochondria and induces apoptosis in H9c2 rat cardiomyoblasts. *J Cell Mol Med* 13(9A), 2926-37 (2009)

12. Peters J., H. Wanka, B. Peters and S. Hoffmann: A renin transcript lacking exon 1 encodes for a non-secretory intracellular renin that increases aldosterone production in transgenic rats. *J Cell Mol Med* 12(4), 1229-37 (2008)

13. Lavoie J. L., X. Liu, R. A. Bianco, T. G. Beltz, A. K. Johnson and C. D. Sigmund: Evidence supporting a functional role for intracellular renin in the brain. *Hypertension* 47(3), 461-6 (2006)

14. Neupert W. and M. Brunner: The protein import motor of mitochondria. *Nat Rev Mol Cell Biol* 3(8), 555-65 (2002)

15. Clausmeyer S., A. Reinecke, R. Farrenkopf, T. Unger and J. Peters: Tissue-specific expression of a rat renin transcript lacking the coding sequence for the prefragment and its stimulation by myocardial infarction. *Endocrinology* 141(8), 2963-70 (2000)

16. Xu D., G. R. Borges, J. L. Grobe, C. J. Pelham, B. Yang and C. D. Sigmund: Preservation of intracellular renin expression is insufficient to compensate for genetic loss of secreted renin. *Hypertension* 54(6), 1240-7 (2009)

17. Oliverio M. I., H. S. Kim, M. Ito, T. Le, L. Audoly, C. F. Best, S. Hiller, K. Kluckman, N. Maeda, O. Smithies and T. M. Coffman: Reduced growth, abnormal kidney structure, and type 2 (AT2) angiotensin receptor-mediated blood pressure regulation in mice lacking both AT1A and AT1B receptors for angiotensin II. *Proc Natl Acad Sci U S A* 95(26), 15496-501 (1998)

18. Takahashi N., M. L. Lopez, J. E. Cowhig, Jr., M. A. Taylor, T. Hatada, E. Riggs, G. Lee, R. A. Gomez, H. S.

Kim and O. Smithies: Ren1c homozygous null mice are hypotensive and polyuric, but heterozygotes are indistinguishable from wild-type. *J Am Soc Nephrol* 16(1), 125-32 (2005)

19. Kim H. S., N. Maeda, G. T. Oh, L. G. Fernandez, R. A. Gomez and O. Smithies: Homeostasis in mice with genetically decreased angiotensinogen is primarily by an increased number of renin-producing cells. *J Biol Chem* 274(20), 14210-7 (1999)

20. Takahashi N., F. Li, K. Hua, J. Deng, C. H. Wang, R. R. Bowers, T. J. Bartness, H. S. Kim and J. B. Harp: Increased energy expenditure, dietary fat wasting, and resistance to diet-induced obesity in mice lacking renin. *Cell Metab* 6(6), 506-12 (2007)

21. Lochard N., D. W. Silversides, J. P. van Kats, C. Mercure and T. L. Reudelhuber: Brain-specific restoration of angiotensin II corrects renal defects seen in angiotensinogen-deficient mice. *J Biol Chem* 278(4), 2184-9 (2003)

22. Erdmann B., K. Fuxe and D. Ganten: Subcellular localization of angiotensin II immunoreactivity in the rat cerebellar cortex. *Hypertension* 28(5), 818-24 (1996)

23. Robertson A. L., Jr. and P. A. Khairallah: Angiotensin II: rapid localization in nuclei of smooth and cardiac muscle. *Science* 172(988), 1138-9 (1971)

24. Re R. N., D. L. Vizard, J. Brown and S. E. Bryan: Angiotensin II receptors in chromatin fragments generated by micrococcal nuclease. *Biochem Biophys Res Commun* 119(1), 220-7 (1984)

25. De Mello W. C.: Influence of intracellular renin on heart cell communication. *Hypertension* 25(6), 1172-7 (1995)

26. Haller H., C. Lindschau, B. Erdmann, P. Quass and F. C. Luft: Effects of intracellular angiotensin II in vascular smooth muscle cells. *Circ Res* 79(4), 765-72 (1996)

27. De Mello W. C.: Intracellular angiotensin II regulates the inward calcium current in cardiac myocytes. *Hypertension* 32(6), 976-82 (1998)

28. Eto K., Y. Ohya, Y. Nakamura, I. Abe and M. Iida: Intracellular angiotensin II stimulates voltage-operated Ca(2+) channels in arterial myocytes. *Hypertension* 39(2 Pt 2), 474-8 (2002)

29. Cook J. L., Z. Zhang and R. N. Re: *In vitro* evidence for an intracellular site of angiotensin action. *Circ Res* 89(12), 1138-46 (2001)

30. Cook J. L., R. Re, J. Alam, M. Hart and Z. Zhang: Intracellular angiotensin II fusion protein alters AT1 receptor fusion protein distribution and activates CREB. *J Mol Cell Cardiol* 36(1), 75-90 (2004)

31. Tadevosyan A., A. Maguy, L. R. Villeneuve, J. Babin, A. Bonnefoy, B. G. Allen and S. Nattel: Nuclear-delimited angiotensin receptor-mediated signaling regulates cardiomyocyte gene expression. *J Biol Chem* 285(29), 22338-49 (2010)

32. Abadir P. M., D. B. Foster, M. Crow, C. A. Cooke, J. J. Rucker, A. Jain, B. J. Smith, T. N. Burks, R. D. Cohn, N. S. Fedarko, R. M. Carey, B. O'Rourke and J. D. Walston: Identification and characterization of a functional mitochondrial angiotensin system. *Proc Natl Acad Sci U S A* 108(36), 14849-54 (2011)

33. Re R. N. and J. L. Cook: The mitochondrial component of intracrine action. *Am J Physiol Heart Circ Physiol* 299(3), H577-83 (2010)

34. Takahashi S., T. Ohsawa, R. Miura and Y. Miyake: Purification of high molecular weight (HMW) renin from porcine kidney and direct evidence that the HMW renin is a complex of renin with renin binding protein (RnBP). *J Biochem* 93(1), 265-74 (1983)

35. Schmitz C., M. Gotthardt, S. Hinderlich, J. R. Leheste, V. Gross, H. Vorum, E. I. Christensen, F. C. Luft, S. Takahashi and T. E. Willnow: Normal blood pressure and plasma renin activity in mice lacking the renin-binding protein, a cellular renin inhibitor. *J Biol Chem* 275(20), 15357-62 (2000)

36. Tan Q., G. De Benedictis, S. V. Ukraintseva, C. Franceschi, J. W. Vaupel and A. I. Yashin: A centenarian-only approach for assessing gene-gene interaction in human longevity. *Eur J Hum Genet* 10(2), 119-24 (2002)

37. Peters J.: Secretory and cytosolic (pro)renin in kidney, heart, and adrenal gland. *J Mol Med (Berl)* 86(6), 711-4 (2008)

38. Peters J., K. Munter, M. Bader, E. Hackenthal, J. J. Mullins and D. Ganten: Increased adrenal renin in transgenic hypertensive rats, TGR(mREN2)27, and its regulation by cAMP, angiotensin II, and calcium. *J Clin Invest* 91(3), 742-7 (1993)

39. Peters J., N. Obermuller, A. Woyth, B. Peters, C. Maser-Gluth, B. Kranzlin and N. Gretz: Losartan and angiotensin II inhibit aldosterone production in anephric rats via different actions on the intraadrenal renin-angiotensin system. *Endocrinology* 140(2), 675-82 (1999)

40. Peters J. and S. Clausmeyer: Intracellular sorting of renin: cell type specific differences and their consequences. *J Mol Cell Cardiol* 34(12), 1561-8 (2002)

Key words: Angiotensin, Renin, Prorenin, Secretory renin, Cytoplasmatic Renin, Intracellular Renin-Angiotensin System, Review

Send correspondence to: Jorg Peters, Institute of Physiology, University of Greifswald, Greifswalder Str. 11C, D-17495 Karlsburg, Germany, Tel: 49 3834 8619309, Fax: 49 3834 8619310, E-mail: joerg.peters@uni-greifswald.de