Kidney International, Vol. 46 (1994), pp. 1522–1524

Proteolytic processing of human prorenin in renal and non-renal tissues

TIMOTHY L. REUDELHUBER, DJAMEL RAMLA, LINDA CHIU, CHANTAL MERCURE, and NABIL G. SEIDAH

Laboratories of Molecular Biochemistry of Hypertension, and Biochemical Neuroendocrinology, Clinical Research Institute of Montreal, Montreal, Quebec, Canada

Proteolytic processing of human prorenin in renal and non-renal tissues. Previous studies have demonstrated that the mouse proprotein convertase PC1 (mPC1) accurately cleaves human prorenin to generate active renin and that this processing event appears to require co-packaging in secretory granules. In the current study, we have tested human PC1 (hPC1; also called PC3) for its ability to activate human prorenin. Our results suggest that while hPC1 is capable of carrying out the specific cleavage of human prorenin, it does so at a reduced efficiency as compared to mPC1. This difference is due to sequences in the carboxy-terminus of PC1 as demonstrated by the activity of hybrid hPC1/mPC1 molecules. These studies demonstrate that PC1 cleavage of prorenin can occur in humans and identify a functionally important region in the hPC1 protein for this interaction. Moreover, the localization of PC1 in human tissues suggests that it may participate in the generation of active renin in the adrenal medulla and possibly in certain adrenal tumors.

Human renin is generated *in vivo* by proteolytic cleavage at a pair of basic amino acids resulting in the removal of a 43 amino acid prosegment from the amino terminus of prorenin [1]. In the juxtaglomerular (JG) cells of the kidney, this activation step occurs subsequent to intracellular targeting of prorenin to dense core secretory granules [2]. Candidate prorenin processing enzymes (PPEs) would be expected, therefore, to cleave prorenin with both site- and organelle-specificity. Previous studies have suggested that both human cathepsin B and mouse PC1 may meet these criteria. Cathepsin B cleaves human prorenin *in vitro* with a high affinity and site-selectivity and has been co-localized with renin by immunocytochemistry in the dense core secretory granules of JG cells and human prolactinomas [3–6]. Using model tissue culture systems, mouse PC1 (mPC1) has also been shown to cleave human prorenin with the expected site-specificity [7]. In addition, this cleavage only takes place in cells containing secretory granules [7]. In the current study, we have tested for the ability of human PC1 to cleave human prorenin in an effort to determine whether it could play the role of a PPE in human tissues.

Methods

Construction of expression vectors was carried out as previously described [7]. Generation of the hybrid hPC1/mPC1 cDNA (h/m HYB1) was carried out by overlap extension PCR. The expressed protein encodes a fusion protein consisting of the signal peptide and first 597 amino acids of hPC1 fused to amino acids 598-726 of mPC1 [8]. Co-transfection of GH_4C1 cells and determination of secreted prorenin and active renin were also carried out as previously described [7]. Briefly, GH_4C1 cells were plated at a density of 5×10^5 cells/well in a six-well dish and were transfected 24 hours later with a calcium phosphate precipitate (300 μl) containing 1.2 μg of an expression vector encoding human prorenin (pRHR1100) and 4.8 μg of the appropriate protease expression vector. Twenty-four hours after transfection, the cells were glycerol shocked and the medium was replaced with 2.0 ml of fresh medium. Sixteen hours later, the medium was collected and assayed for active renin and prorenin (trypsin-activatable renin less active renin) using the angiotensin I generation assay.

Results

As previously reported [7], co-transfection of GH_4C1 cells with an expression vector encoding human prorenin and a neutral plasmid (pUC18) leads to secretion of predominantly inactive prorenin (Fig. 1, Cont.). However, when the human prorenin expression vector is co-transfected with a vector encoding hPC1, approximately 12% of the prorenin is converted to active renin (Fig. 1, Native). Human prorenin containing mutations in either the Lys or Arg residues at the cleavage site are not converted to active renin by hPC1 (Fig. 1, K/A-2 and R/A-1). These results suggest that hPC1, like mPC1, is capable of activating human prorenin by specific cleavage at the pair of basic residues previously reported for cleavage in the human kidney [1].

Co-transfection of GH_4C1 cells with the human prorenin expression vector and either hPC1 or mPC1 (Fig. 2) reveals that mPC1 consistently generated more active human renin than hPC1 (31.2% vs. 12.3%, respectively, $P < 0.001$). Even though comparison of the cDNAs encoding hPC1 and mPC1 reveals that the two proteases share an overall protein sequence homology of 92.6% [8] amino acids 598-619 only display a 36% identity. To determine whether the carboxy-terminal sequence differences between hPC1 and mPC1 might be responsible for the apparent difference in prorenin processing activity, a hybrid PC1 cDNA was constructed which encodes the amino terminal 597 amino acids of hPC1 linked to the carboxy-terminus (amino acids 598-726) of mPC1 (Fig. 2A, h/m HYB1). Co-expression of this hybrid PC1 with human prorenin leads to activation of human prorenin at an efficiency comparable to that of mPC1 (26.2%; Fig. 2B). These results suggest that the apparent human prorenin processing activity

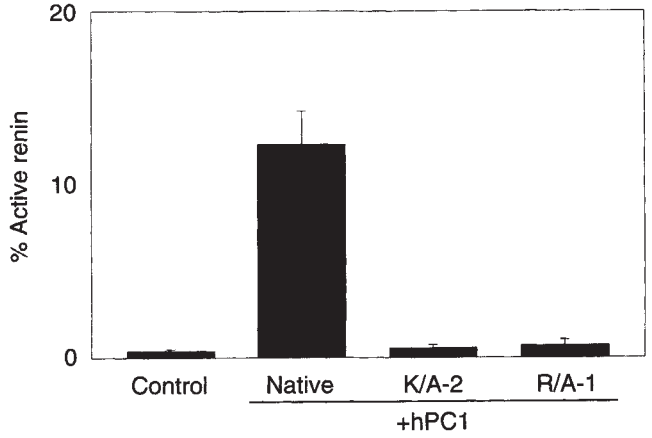

Fig. 1. *Human PC1 accurately processes human prorenin.* Expression vectors for either native human prorenin (Cont. and Native) or human prorenin mutated at the native cleavage site (K/A-2 and R/A-1) were co-transfected into GH_4C1 cells with either a control plasmid (pUC18; Cont.) or an expression vector for hPC1 (Native, K/A-2 and R/A-1). Shown is the percent active renin (active renin/total renin) secreted. Results represent the mean ±SEM of 4 independent transfections.

differences between mPC1 and hPC1 are due to amino acids in the carboxy-terminal 1/5 of the respective proteases.

Discussion

Significant differences exist in the processing events of prorenins from different tissues and species. Processing of prorenin in the mouse submaxillary gland (SMG) occurs at the analogous position to that of human renin [9], but processing of rat renal renin seems to occur after a threonine residue which is located 7 amino acids toward the carboxy terminus relative to the human renin processing site [10, 11]. The proteolytic cleavage site of mouse renal prorenin is currently unknown. Rat renal renin and mouse SMG renin (the product of the *Ren-2* gene) subsequently undergo an additional internal processing event, converting "one-chain" active renin to a "two-chain" molecule [9–11] in which the two halves are held together by a disulfide bridge. It is not yet known whether mouse renal renin also undergoes a similar secondary cleavage. Notably, while renal renins appear to be processed within secretory granules, mouse SMG renin may be processed to "one-chain" renin within the Golgi apparatus and only a portion of the protein is further processed in granules to yield the "two-chain" protein [12]. Recently, two kallikrein-like enzymes capable of processing mouse SMG prorenin have been isolated from submaxillary glands [13, 14]. Notably, while these enzyme are able to generate "one-chain" renin, they are unable to carry out the second cleavage to yield the "two-chain" form [13]. In addition, kidney glandular kallikrein cannot activate mouse SMG prorenin and PRECE (one of the SMG convertases) is unable to activate mouse renal (*Ren-1*) or human prorenins [13]. Indeed, as of this date, there have been no published reports of enzymes capable of activating either mouse renal prorenin (*Ren-1*) or rat prorenin. Thus, while renins from mice, rats and humans share many similarities in protein structure and function, differences may exist in the way they are activated by processing enzymes in different tissues.

PC1 represents a candidate PPE with two important characteristics: the ability to carry out specific cleavage of human prorenin and a seeming requirement for secretory granules in this processing event. When tested in a similar co-transfection assay, human cathepsin B has not demonstrated the ability to cleave human prorenin (T.L. Reudelhuber and Chantal Mercure, unpublished observation). Could PC1 be the renal prorenin processing enzyme? It seems unlikely for two reasons: First, we have been unable to detect immunoreactivity for hPC1 in human JG cells although it does appear to be expressed in the adrenal medulla where it may participate in prorenin activation in normal tissue and in derived tumors such as pheochromocytomas (D. Ramla, T. Reudelhuber M. Marcinciewicz and N. Seidah, submitted for publication). Second, while mPC1 is able to cleave human prorenin and mouse SMG prorenin, it cannot activate mouse or rat renal prorenin [7, 15]. Could the PPE in rodent and primate kidneys differ? Injection of either mouse [16, 17] or rat [18] embryos with the intact human prorenin gene results in the generation of transgenic animals with active human renin in their circulation. Thus, in spite of the apparent species selectivity of PPE activities currently reported *in vitro*, mouse rat and human kidneys should all express an enzyme(s) capable of activating human prorenin. The role of cathepsin B and/or other endoproteases in the renal processing of prorenin remains an important area of future investigation.

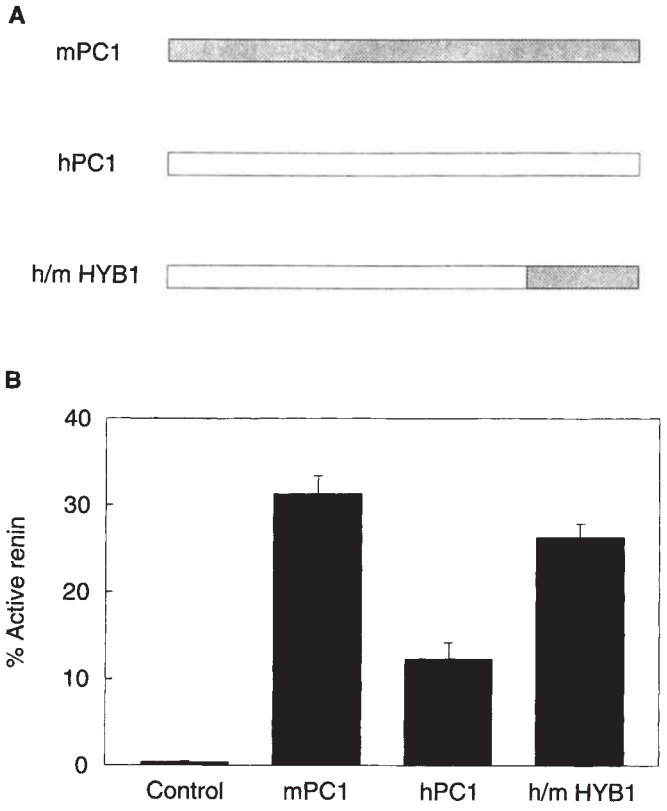

Fig. 2. *Differences in cleavage efficiencies of mPC1 and hPC1.* **(A)** Schematic representation of the PC1 proteins co-expressed with human prorenin in GH_4C1 cells. The hybrid PC1 (h/m HYB1) encodes aa 1-597 of hPC1 and aa 598-619 of mPC1. **(B)** Relative ability of the various PC1 proteins to cleave human prorenin. Results represent the mean ±SEM of 4 independent transfections.

Acknowledgment

This work was supported by a Group grant from the Medical Research Council of Canada to the Multidisplinary Research Group on Hypertension.

Reprint requests to Timothy L. Reudelhuber, Ph.D., IRCM, 110, Pine Ave, West, Montreal, Quebec, H2W 1R7 Canada.

References

1. Do YS, Shinagawa T, Tam H, Inagami T, Hsueh WA: Characterization of pure human renal renin. *J Biol Chem* 262:1037–1043, 1987
2. Taugner R, Kim SJ, Murakami K, Waldherr R: The fate of prorenin during granulopoiesis in epithelioid cells. Immunocytochemical experiments with antisera against renin and different portions of the renin prosegment. *Histochemistry* 86:249–253, 1987
3. Wang PH, Do YS, Macaulay L, Shinagawa T, Anderson FW, Baxter JD, Hsueh WA: Identification of renal cathepsin B as a human prorenin-processing enzyme. *J Biol Chem* 266:12633–12638, 1991
4. Taugner R, Hackenthal E: On the character of the secretory granules in juxtaglomerular epithelioid cells. *Int Rev Cyto* 110:93–131, 1988
5. Matsuba H, Watanabe T, Watanabe M, Ishii Y, Waguri S, Kominami E, Uchiyama Y: Immunocytochemical localization of prorenin, renin, and cathepsins B, H, and L in juxtaglomerular cells of rat kidney. *J Histochem Cytochem* 37:1689–1697, 1989
6. Saint-Andre JP, Rohmer V, Pinet F, Rousselet MC, Bigorgne JC, Corvol P: Renin and cathepsin B in human pituitary lactotroph cells. An ultrastructural study. *Histochemistry* 91:291–297, 1989
7. Benjannet S, Reudelhuber T, Mercure C, Rondeau N, Chrétien M, Seidah NG: Pro-protein conversion is determined by a multiplicity of factors including convertase processing, substrate specificity and intracellular environment. *J Biol Chem* 267:11417–11423, 1992
8. Seidah NG, Hamelin J, Gaspar AM, Day R, Chretien M: The cDNA sequence of the human pro-hormone and pro-protein convertase PC1. *DNA Cell Biol* 11:283–289, 1992
9. Misono KS, Chang J, Inagami T: Amino acid sequence of mouse submaxillary gland renin. *Proc Natl Acad Sci USA* 79:4858–4862, 1982
10. Kim S, Hosoi M, Kikuchi N, Yamamoto K: Amino-terminal amino acid sequence and heterogeneity in glycosylation of rat renal renin. *J Biol Chem* 266:7044–7050, 1991
11. Campbell DJ, Valentijn AJ, Condron R: Purification and amino-terminal sequence of rat kidney renin: Evidence for a two-chain structure. *J Hypertens* 9:29–33, 1991
12. Pratt RE, Ouellette AJ, Dzau VJ: Biosynthesis of renin: Multiplicity of active and intermediate forms. *Proc Natl Acad Sci USA* 80:6809–6813, 1983
13. Nakayama K, Kim W-S, Nakagawa T, Nagahama M, Murakami K: Substrate specificity of prorenin converting enzyme of mouse submandibular gland. *J Biol Chem* 265:21027–21031, 1990
14. Kim WS, Nakayama K, Murakami K: The presence of two types of prorenin converting enzymes in the mouse submandibular gland. *FEBS Lett* 293:142–144, 1991
15. Nagahama M, Nakayama K, Murakami K: Sequence requirements for prohormone processing in mouse pituitary AtT-20 cells: Analysis of prorenins as model substrates. *Eur J Biochem* 197:135–140, 1991
16. Sigmund CD, Jones CA, Kane CM, Wu C, Lang JA, Gross KW: Regulated tissue- and cell-specific expression of the human renin gene in transgenic mice. *Circ Res* 70:1070–1079, 1992
17. Fukamizu A, Sugimura K, Takimoto E, Sugiyama F, Seo MS, Takahashi S, Hatae T, Kajiwaran N, Yagami K, Murakami K: Chimeric renin-angiotensin system demonstrates sustained increase in blood pressure of transgenic mice carrying both human renin and human angiotensinogen genes. *J Biol Chem* 268:11617–11621, 1993
18. Ganten D, Wagner J, Zeh K, Bader M, Michel JB, Paul M, Zimmermann F, Ruf P, Hilgenfeldt U, Ganten U, et al: Species specificity of renin kinetics in transgenic rats harboring the human renin and angiotensinogen genes. *Proc Natl Acad Sci USA* 89:7806–7810, 1992