REGION-SPECIFIC RADIOIMMUNOASSAY SYSTEMS FOR HUMAN PRORENIN AND RENIN WITH USE OF SYNTHETIC PEPTIDES

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ABSTRACT

Two specific radioimmunoassay systems for human prorenin and prorenin/renin were developed using polyclonal antibodies R0066 and R0417 raised against synthetic peptides corresponding to the 21-64 and 337-360 sequences of human preprorenin, respectively. The assay system with antibody R0066 was proved to recognize the amino-terminal extremity of the propeptide of prorenin, and the other system with antibody R0417 recognized mainly the amino-terminal portion of the 337-360 sequence and was supposed to crossreact with both prorenin and renin. The specificities of the two radioimmunoassay systems were confirmed by examining prorenin/renin components in human kidney extracts using the assay systems and renin enzyme activity assay combined with gel filtration and DEAE-HPLC. The assay system with antibody R0066 was considered to be of special value for detecting the intact, but not truncated, molecule of prorenin. Thus, the combined use of the two radioimmunoassays provided a measure to discriminate renin and prorenin. These radioimmunoassay systems will be advantageously utilized in the characterization of prorenin/renin and related proteins in human tissues and biological fluids.

The renin-angiotensin system is known to be a key regulator of blood pressure and fluid and electrolyte balance. Renin is an aspartyl proteinase that acts on angiotensinogen to generate the decapeptide angiotensin I (24, 25). The kidney has been accepted as a major source of the enzyme. However, the presence of the enzyme in extrarenal tissues has also been well documented in humans and various animal species. In human tissues, there is one copy of the renin gene (6, 10, 19, 22), while heterogeneity in molecular forms of the protein products has been described (for reviews, see 11, 13, 16, 20, 21), although the molecular features of the products have not always been fully characterized.

We have previously succeeded in the production of antibodies specific for human prorenin and prorenin/renin, using synthetic peptides corresponding to the 21-64 (T-F-G-L-P-T-D-T-T-F-K-R-I-F-L-K-R-M-P-S-I-R-E-S-L-K-E-R-G-V-D-M-A-R-L-G-

P-E-W-S-Q-P-M) and 337-360 (H-L-G-G-K-E-Y-T-L-T-S-A-D-Y-V-F-Q-E-S-Y-S-S-K-K) sequences of human renal preprorenin, respectively (33) (Fig. 1). Both antibodies were shown to be useful for immunohistochemical demonstration of prorenin and prorenin/renin in human kidney. This was the first case of the production of antibody, with use of synthetic peptide, having an antigenic determinant within renin molecule. The aim of the present study was to develop radioimmunochemical methods using anti-synthetic peptide antibodies for molecular characterization of prorenin/renin (inactive/active renin) and related proteins in human tissues and biological fluids.

MATERIALS AND METHODS

Tissue Extraction

Normal renal tissue specimens (approximately 1 g each) were obtained at surgery of renal cancer

Fig. 1 Schematic presentation of human preprorenin, prorenin and renin with antigens used for production of antibodies R0066 and R0417

patients: specimen A from a male patient (H. S.) of age 54 and specimen B from a male patient (Y. S.) of age 73. Informed consents had been obtained from the patients prior to operation. The tissue specimens were immediately frozen. Each of the tissues was homogenized with a Bio-Mixer Model BM-1 in 5 ml of ice-cold 50 mM Tris-HCl buffer (pH 7.4) containing 5% glycerol, 1 mM phenylmethylsulfonylfluoride (PMSF) (Sigma Chemicals, St. Louis, MO, U.S.A.) and 200 KIU/ml aprotinin (Bayer, Leverkusen, Germany) in an ice bath. The homogenate was centrifuged at $10,000 \times g$ for 30 min at 4°C and the supernatant was taken and stored at -30°C . Protein concentration in each extract was determined by the method of Bradford (2).

Gel Filtration

Extract of kidney tissue specimen A (2 ml) was mixed with 1 ml of 50 mM citrate buffer (pH 5.0) containing 50 mM NaCl and 25 mM EDTA. The mixture was applied to a column (1.6×100 cm) of Sephadex G-150 superfine and the column was eluted with the same buffer. Eluates (2 ml each) were collected and each fraction was divided into three portions, which were then submitted to the two radioimmunoassays and renin enzyme activity assay, respectively.

DEAE-HPLC

Extract of kidney tissue specimen B was diluted with a twofold volume of 20 mM phosphate buffer (pH 4.5). The mixture was filtered through microfilter (Nihon Millipore, Tokyo) and the filtrate was diluted with 1 ml of 20 mM phosphate buffer (pH 6.3). The sample (10 mg protein) was applied to a column (7.5×75 mm) of TSK-Gel DEAE-3SW (Tosoh, Tokyo) attached to a Toyosoda HLC-803D (Tosoh, Tokyo). The column was eluted with 20

mM phosphate buffer (pH 6.3) for 10 min at a flow rate of 1 ml/min, followed by a linear gradient of NaCl from 0 to 1 M in 20 mM phosphate buffer (pH 6.3) for 50 min. Fractions (1 ml each) were collected and each fraction was divided into three portions, which were then submitted to the two radioimmunoassays and renin enzyme activity assay, respectively.

Radioimmunoassays

Prorenin radioimmunoassay Antibody R0066 was raised against a synthetic peptide corresponding to the 21–64 sequence of human preprorenin, hPPR(21–64), as described previously (33). Since neither tyrosine nor histidine residue is present in hPPR(21–64), 3-(4-hydroxyphenyl)propionyl-hPPR(21–64) [HPP-hPPR(21–64)] was synthesized by the solid phase methodology and used for radioiodination. The HPP-hPPR(21–64) was radioiodinated by the chloramine T method and the labeled peptide was purified on a Sephadex G-10 column with 1 M acetic acid as eluent. Synthetic hPPR(21–64) was used as standard antigen. The standard diluent used for the assay was 50 mM citrate buffer (pH 5.0) containing 50 mM NaCl, 25 mM EDTA and 0.5% bovine serum albumin (BSA). To each of assay tubes, 0.1 ml of standard peptide or sample, 0.1 ml of diluted ($\times 10,000$) rabbit anti-hPPR(21–64) serum (R0066) and 0.1 ml of ^{125}I -HPP-hPPR(21–64) ($\approx 5,000$ cpm) were added. The mixture was incubated at 4°C for 48 h, to which were added 0.1 ml of diluted ($\times 100$) normal rabbit serum (Daiichi Radioisotope Laboratories, Tokyo), 0.1 ml of a twentyfold diluted goat anti-rabbit IgG serum (Daiichi Radioisotope Laboratories, Tokyo) and 0.9 ml of 5% (w/v) polyethylene glycol 6000. The mixture was further incubated at 4°C for 6 h and then centrifuged at $2,000 \times g$ at 4°C for 30 min. The supernatant was aspirated and the precipitate was counted in a gamma counter.

Fig. 2 Typical standard curves (—●—) of the prorenin (a) and prorenin/renin (b) radioimmunoassays with dilution curves (—▲—) of human kidney (specimen A) extract in the assays

Prorenin/renin radioimmunoassay Antibody R0417 was produced against a synthetic peptide corresponding to the 337–360 sequence of human preprorenin, hPPR(337–360), as described previously (33). Labeled antigen was prepared by radioiodination of synthetic hPPR(337–360) by the chloramine T method, followed by gel filtration on a Sephadex G-10 column using 1 M acetic acid as eluent. Synthetic hPPR(337–360) was used as standard antigen. Radioimmunoassay was carried out in the same manner as that employed for the prorenin radioimmunoassay. Antibody R0417 was used at a final dilution of 1:7,000.

Renin Enzyme Activity Assay

Renin enzyme activity was measured according to the method of Ikeda and coworkers (12), with slight modifications using RENIN·RIABEAD (Dainabot, Tokyo). Briefly, to each test tube were added 0.02 ml of 0.6 M citrate buffer containing 10 mM EDTA, 0.1 ml of unknown sample, 0.01 ml of a stock solution of PMSF in ethanol (10 mg/ml) and 0.1 ml of a fivefold diluted human plasma with saline as substrate. The mixtures were incubated at 37°C for 1 h and then 0.1 ml each of a solution of ^{125}I -angiotensin I ($\approx 40,000$ cpm) containing 0.5 mg/ml pepstatin A was added on cooling in an ice bath. Anti-angiotensin I antibody-coated balls were placed in all test tubes and the tubes were kept for 3 h at room temperature, when 1 ml each of distilled water was added. The solution was

aspirated, and the balls in tubes were washed with 1 ml of distilled water and counted in a gamma counter.

Gel Electrophoresis and Immunoblotting

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting were carried out according to the methods of Laemmli (18) and Towbin and coworkers (31), respectively, with minor modifications. Briefly, sample was boiled at 100°C for 2 min in 62.5 mM Tris-HCl buffer (pH 6.8) containing 2% SDS, 10% glycerol and 5% 2-mercaptoethanol, which was then subjected to electrophoresis on 12.5% polyacrylamide slab gel (Daiichi Pure Chemicals, Tokyo) at 40 mA for 1.5 h in electrode buffer (pH 8.3) comprising 25 mM Tris-HCl, 192 mM glycine and 0.1% SDS. The gel was then transferred, at 10 V/cm for 2 h, to nitrocellulose membrane (0.45 μm) (Pharmacia LKB Biotechnology, Uppsala, Sweden) in 25 mM Tris-HCl buffer (pH 8.3) containing 192 mM glycine and 10% methanol. The membrane was processed for immunodetection with a diluted ($\times 500$) anti-hPPR(21–64) serum R0066 or anti-hPPR(337–360) serum R0417 using a commercial kit for immunoblot (Amersham International plc, Amersham, U.K.).

RESULTS

Fig. 2, a and b show typical standard curves for

Table 1 *Characterization of Radioimmunoassays with Use of Antibodies R0066 and R0417*

Peptides	Relative crossreactivities of peptides (%)	
	Radioimmunoassay with antibody R0066	Radioimmunoassay with antibody R0417
hPPR(21-64)	accepted as 100	≈ 0
hPPR(34-64)	0.1	—
hPPR(56-64)	≈ 0	—
hPPR(337-360)	≈ 0	accepted as 100
hPPR(344-360)	—	5

Relative crossreactivities were estimated by comparing the amounts of the peptide and standard hPPR(21-64) or hPPR(337-360) required for 50% inhibition of antibody binding of the tracer.

radioimmunoassays for prorenin and prorenin/renin, respectively, together with dilution curves of human kidney extract (specimen A) in the assay systems. The standard curves were both linear in logit-log scale and the dilution curves paralleled the standard curves in the respective assay systems. The minimum detectable dose was about 10 fmol in either system. No crossreaction was detected in either assay system with bovine spleen cathepsin D (Sigma Chemicals, St. Louis, MO, U.S.A.) in doses up to 100 ng. Table 1 indicates the characterization of the two assay systems with antibodies R0066 and R0417, respectively, using synthetic prorenin/renin related peptides. The prorenin assay system with antibody R0066 was shown to recognize predominantly the amino terminal portion of the propeptide, and the prorenin/renin assay system with antibody R0417 recognized the amino terminal portion of the 337-360 sequence of human preprorenin.

Immunoreactivities measured by the prorenin assay system (PR-IR) and by the prorenin/renin assay system (PR/R-IR) were 0.7 and 0.5 pmol/mg protein in the crude extract of specimens A and 1.0 and 2.3 pmol/mg protein in specimen B extract, respectively. Relative contents of PR/R-IR to PR-IR in other human kidney extracts also varied depending on samples examined (data not shown), and specimens A and B contained rather high concentrations of PR/R-IR, which were purposely used for the present chromatographic analyses.

The gel filtration profiles of PR-IR and PR/R-IR in the extract of kidney tissue specimen A are shown in Fig. 3, a and b with the elution profile of renin enzyme activity (REA) (Fig. 3c). The analytical recoveries of PR-IR and PR/R-IR in gel filtration were 81% and 75%, respectively. The peaks of

PR-IR and renin immunoreactivity (R-IR) could not be separated sufficiently in this gel filtration (Fig. 3b). However, the latter portion of the PR/R-IR peak (fraction numbers 53-58) in Fig. 3b had little PR-IR and corresponded to the peak of REA in Fig. 3c, indicating the elution of R-IR in the positions. No small molecular forms having PR-IR or/and R-IR were detected.

SDS-PAGE followed by immunoblot analysis of the peak fraction of PR-IR and PR/R-IR in gel filtration showed a band corresponding to 49,000 Da with antibody R0066 and two bands of 49,000 and 40,000 Da with antibody R0417.

The extract of kidney tissue specimen B was analyzed by DEAE-HPLC. Fig. 4, a-c demonstrate the elution profiles of PR-IR, PR/R-IR and REA. The two radioimmunoassays well distinguished the peaks of prorenin and renin components. Peak I (fraction number 12) showed both PR-IR and PR/R-IR without REA, while peaks II and III (fraction numbers 28 and 30, respectively) had PR/R-IR and REA with little PR-IR, indicating that peak I component represents prorenin or its related protein, while peaks II and III components are renin or its related proteins. The molar ratio of PR-IR and PR/R-IR in peak I component was 1:0.61. As can be seen in Fig. 4a, small PR-IR peaks also appeared in fractions 30 and 34, which overlapped to large peaks (II and III) of PR/R-IR (Fig. 4b) and REA (Fig. 4c). Peak IV (fraction number 33) was found to have a high REA as compared with PR/R-IR in the fraction.

DISCUSSION

Immunochemical manipulations using specific

Fig. 3 Gel filtration profiles of PR-IR (a) and PR/R-IR (b) in human kidney (specimen A) extract measured by the prorenin and prorenin/renin radioimmunoassays, respectively, and renin enzyme activity (REA) in the gel-filtration fractions (c)

monoclonal or polyclonal antibodies are now widely used for characterization and quantification of biologically active principles. Synthetic peptides are useful as haptenic immunogens for production of region-specific antibodies against a biological peptide or protein of interest. We have previously proved the advantage of the use of synthetic peptides as immunogens without conjugated carrier macromolecules (32, 34–36). The currently used antibodies specific for human prorenin and renin were also raised against synthetic peptides, hPPR (21–64) and (337–360), respectively (Fig. 1), and they have been proved to be useful in immuno-histochemistry of prorenin/renin in human kidney (33). hPPR(21–64) contains the full sequence of

Fig. 4 DEAE-HPLC profiles of PR-IR (a) and PR/R-IR (b) in human kidney (specimen B) extract measured by the prorenin and prorenin/renin radioimmunoassays, respectively, and renin enzyme activity (REA) in the chromatographic fractions (c)

the propeptide of prorenin except the carboxy-terminal two basic amino acid residues Lys⁶⁵-Arg⁶⁶. In the present study, polyclonal anti-prorenin antibody R0066 was shown to recognize predominantly the amino-terminal region of the propeptide, and the radioimmunoassay with use of the antibody was proved to be useful for detection of prorenin molecule in human kidney extract. Day and coworkers (3) have obtained a human prorenin amino-terminal specific monoclonal antibody using a synthetic peptide corresponding to the amino-ter-

minal nonapeptide sequence of prorenin, but the antibody was found to crossreact with inactive renin (prorenin) only after denaturation. Other attempts to produce antibodies specific for the amino-terminal portion of the propeptide with use of synthetic peptides corresponding to the full or amino-terminal partial sequence of the propeptide have not been succeeded (3, 15, 27, 28). Productions of anti-synthetic peptide antibodies against the propeptide of human prorenin have also been carried out by others (1, 5, 9, 17, 23, 27, 28), but the peptides used in these studies as haptenic immunogens were short peptides corresponding to the carboxy-terminal or middle regions of the propeptide. On the other hand, the presence of the amino-terminal truncated forms of prorenin has been described (7, 15, 29, 30), although full consensus has not yet been obtained (21, 26). Pratt and coworkers (26) have provided data suggesting that prorenin is secreted from the kidney in the intact form and not as a truncated form as proposed by Hirose and coworkers (9). These proposed truncated forms crossreact with antibodies directed to carboxy-terminal and/or middle portions of the propeptide, but not with amino-terminal specific prorenin antibodies. In this context, the present radioimmunoassay with the amino-terminal extremity specific anti-prorenin antibody is of special value for detecting the intact molecule of prorenin and related components having the full sequence of the propeptide. In fact, the radioimmunoassay recognized only one molecular form of 49,000 Da in human kidney extract, which molecular size is consistent with that of human renal prorenin previously reported (14, 20).

Renin is a member of aspartyl proteinases including pepsin, chymosin and cathepsin D. These enzymes show considerable homology in their amino acid sequences (19). A putative three-dimensional structure model of prorenin deduced from the known structure of pepsinogen revealed that the carboxy-terminal half of the propeptide contains α -helical and β -stranded structures, while the amino-terminus extends from the β -strand (7, 11). It was also proposed that the carboxy-terminal portion of the propeptide, i.e. the 42–62 sequence, is buried, whereas the amino-terminal is exposed (8, 21). These stereo-structural features of human prorenin again validate the use of the current polyclonal anti-prorenin antibody R0066 that recognizes the extreme amino-terminal part of the propeptide.

As described in our previous paper (33), the

337–360 sequence of human preprorenin has purposely been chosen for synthesis of a peptide which was used as immunogen for the production of antibodies specific for prorenin/renin, since the sequence homology with other members of aspartyl proteinases in this region is less as compared with other regions of the molecule. We have thus succeeded for the first time in the production of an antibody directed to renin molecule by using synthetic peptide as immunogen. However, anti-hPPR(337–360) antibody R0417 is supposed to crossreact with prorenin as well (Fig. 1). Thus, the combined use of two radioimmunoassays with antibodies R0066 and R0417 was expected to distinguish prorenin and renin. Indeed, this was shown by analysis of human kidney extract with the two radioimmunoassays and renin enzyme assay combined with gel filtration and DEAE-HPLC. The crossreactivity of the prorenin component in the prorenin/renin radioimmunoassay with antibody R0417 was less (61%) than that in the prorenin radioimmunoassay with antibody R0066, presumably because of the steric environment of the antigenic determinant (the 337–360 sequence) in prorenin molecule.

During the purification of human renal renin, Do and coworkers (4) have found the existence of two components, Mr = 22,000 and 18,000, which correspond to the amino- and carboxy-terminal portions of renin, Mr = 44,000, resulting from the cleavage between -Asn²³⁴-Ser²³⁵-(human renin -Asn¹⁶⁸-Ser¹⁶⁹-). The current antibody R0417 is expected to crossreact with the Mr = 18,000 component, if any. However, we could not observe the existence of such a component either in gel filtration or in SDS-PAGE-immunoblot analysis of human kidney extracts.

In the present study, we developed two radioimmunoassay systems which, in combination, could identify prorenin having the full length of the propeptide, as well as renin, in human kidney. The assay system will advantageously be utilized in the characterization of prorenin/renin and related proteins in human extrarenal tissues and biological fluids.

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REFERENCES

1. ATLAS S. A., CHRISTOFALO P., HESSON T., SEALEY J. E. and FRITZ L. C. (1985) Immunological evidence that inactive renin is prorenin. *Biochem. Biophys. Res. Commun.* **132**, 1038–1045

2. BRADFORD M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248–254
3. DAY R. P., HUI K. Y., GURE M., CARLSON W. D., DZAU V. J. and HABER E. (1986) A monoclonal antibody specific for the amino terminal sequence of human prorenin identifies a common epitope on renal and amniotic fluid inactive renins. *J. Hypertens.* **4**, 375–381
4. DO Y.-S., SHINAGAWA T., TAM H., INAGAMI T. and HSUEH W. A. (1987) Characterization of pure human renal renin: evidence for a subunit structure. *J. Biol. Chem.* **262**, 1037–1043
5. GAILLARD I., FEHRENTZ J. A., SIMON D., BADOUILLE G., SEYER R., CASTRO B., PAU B. and CORVOL P. (1986) Analysis of inactive renin by renin profragment monoclonal antibodies. *FEBS Lett.* **207**, 100–104
6. HARDMAN J. A., HORT Y. J., CATANZARO D. F., TELLAM J. T., BAXTER J. D., MORRIS B. J. and SHINE J. (1984) Primary structure of the human renin gene. *DNA* **3**, 457–468
7. HEINRIKSON R. L., HUI J., ZÜRCHER-NEELY H. and POORMAN R. A. (1989) A structural model to explain the partial catalytic activity of human prorenin. *Amer. J. Hypertens.* **2**, 367–380
8. HEINRIKSON R. L. and POORMAN R. A. (1990) Biochemistry and molecular biology of recombinant human renin and prorenin. In *Hypertension: Pathophysiology, Diagnosis, and Management* (ed. LARAGH J. H. and BRENNER B. M.) Raven Press, New York, pp. 1179–1196
9. HIROSE S., KIM S.-J., MIYAZAKI H., PARK Y.-S. and MURAKAMI K. (1985) In vitro biosynthesis of human renin and identification of plasma inactive renin as an activation intermediate. *J. Biol. Chem.* **260**, 16400–16405
10. HOBART P. M., FOGLIANO M., O'CONNOR B. A., SCHAEFER I. M. and CHIRGWIN J. M. (1984) Human renin gene: structure and sequence analysis. *Proc. Natl. Acad. Sci. USA* **81**, 5026–5030
11. HSUEH W. A. and BAXTER J. D. (1991) Human prorenin. *Hypertension* **17**, 469–479
12. IKEDA I., INUMA K., TAKAI M., YANAGAWA Y., KURATA K., OGIHARA T. and KUMAHARA Y. (1981) Measurement of plasma renin activity by a simple solid phase radioimmunoassay. *J. Clin. Endocrinol. Metab.* **54**, 423–429
13. INAGAMI T. (1991) Purification of renin and prorenin. *Hypertension* **18**, 241–251
14. INAGAMI T., CHANG J. J., DYKES C. W., TAKII Y., KISARAGI M. and MISONO K. S. (1983) Renin: structural features of active enzyme and inactive precursor. *Federation Proc.* **42**, 2729–2734
15. ISHIZUKA Y., IIZUMI S., AKIYAMA F., HORI H., POORMAN R. A., LOBL T. J. and MURAKAMI K. (1989) Characterization of monoclonal antibodies against human prorenin profragment and identification of active prorenins in plasma. *J. Biochem.* **106**, 430–435
16. KATZ S. A. and OPSAHL J. A. (1995) Biochemistry and physiology of multiple renin forms. In *Hypertension: Pathophysiology, Diagnosis, and Management* Second Ed. (ed. LARAGH J. H. and BRENNER B. M.) Raven Press, New York, pp. 1489–1502
17. KIM S.-J., HIROSE S., MIYAZAKI H., UENO N., HIGASHIMORI K., MORINAGA S., KIMURA T., SAKAKIBARA S. and MURAKAMI K. (1985) Identification of plasma inactive renin as prorenin with a site-directed antibody. *Biochem. Biophys. Res. Commun.* **126**, 641–645
18. LAEMMLI U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680–685
19. MIYAZAKI H., FUKAMIZU A., HIROSE S., HAYASHI T., HORI H., OHKUBO H., NAKANISHI S. and MURAKAMI K. (1984) Structure of the human renin gene. *Proc. Natl. Acad. Sci. USA* **81**, 5999–6003
20. MORRIS B. J. (1986) New possibilities for intracellular renin and inactive renin now that the structure of the human renin gene has been elucidated. *Clin. Sci.* **71**, 345–355
21. MORRIS B. J. (1992) Molecular biology of renin I: gene and protein structure, synthesis and processing. *J. Hypertens.* **10**, 209–214
22. MORRIS B. J., CATANZARO D. F., HARDMAN J., MESTEROVIC N., TELLAM J., HORT Y. and SHINE J. (1984) Human renin gene sequence, gene regulation and prorenin processing. *J. Hypertens.* **2**, Suppl. 3, 231–233
23. NARUSE K., NARUSE M., HIROSE S., NAGANO M., YAMAKADO M., KURIMOTO F., SAKURAI H., ZENG Z.-P., DEMURA H., INAGAMI T. and SHIZUME K. (1988) Immunoreactive prorenin and its profragment peptide are present in human juxtaglomerular cells. *J. Hypertens.* **6**, Suppl. 4, s426–s428
24. PEACH M. J. (1977) Renin-angiotensin system: biochemistry and mechanisms of action. *Physiol. Rev.* **57**, 313–370
25. PEART W. S. (1978) Renin 1978. *Johns Hopkins Med. J.* **143**, 193–206
26. PRATT R. E., CARLETON J. E., RICHIE J. P., HEUSSER C. and DZAU V. J. (1987) Human renin biosynthesis and secretion in normal and ischemic kidneys. *Proc. Natl. Acad. Sci. USA* **84**, 7837–7840
27. PRICE-JONES M. J., CHARLTON P. A., BESSANT C. M., HARRISON T. M., DARKE B. M., LEES W. E. and KAY J. (1993) Analysis of latent forms of renin using antibodies raised against the propart segment of human prorenin: validation with representative samples of ovarian cyst and follicular fluids. *Clin. Exper. Hypertens.* **15**, 619–640
28. SCHUMACHER M., NANNINGA A., DELFS T., MUKHOPADHYAY A. K. and LEIDENBERGER F. A. (1992) A direct immunoradiometric assay for human plasma prorenin: concentrations in cycling women and in women taking oral contraceptives. *J. Clin. Endocrinol. Metab.* **75**, 617–623
29. SHINAGAWA I., DO Y.-S., BAXTER J., SHINE J. and HSUEH W. A. (1987) Identification of "intermediate" forms of human renin. *Clin. Res.* **35**, 449A
30. TAKADA Y., MARUTA H., WAGNER B., XIANG-GUANG F., HALL J. and ERDÖS E. G. (1987) Prorenin activation by human neutrophil elastase. *Hypertension* **10**, 368
31. TOWBIN H., STAEBELIN T. and GORDON J. (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA* **76**, 4350–4354
32. TOYOSHIGE M., YANAIHARA C., HOSHINO M., KANEKO T. and YANAIHARA N. (1989) Insulin receptor in human hepatoma PLC/PRF/5 cells and its immunochemical characterization with anti-synthetic peptide antibodies. *Biomedical Res.* **10**, 139–147
33. YANAIHARA C., KADOWAKI M., HOSHINO M., KISHIDA S., SUZUKI T. and YANAIHARA N. (1987) Production of region-specific antisera to human prorenin and renin using synthetic peptides. *Biomedical Res.* **8**, 95–101
34. YANAIHARA N., SAKAGAMI M., SATO H., YAMAMOTO K., HASHIMOTO T., YANAIHARA C., ITO Z., YAMAGUCHI K. and

- ABE K. (1977) Immunological aspects of secretin, substance P, and VIP. *Gastroenterology* **72**, 803–810
35. YANAIHARA N., YANAIHARA C., HOSHINO M., MOCHIZUKI T. and IGUCHI K. (1988) Immunochemical and biochemical properties of purposely designed synthetic peptides. *Ann. N.Y. Acad. Sci.* **527**, 29–43
36. YANAIHARA N., YANAIHARA C., NOKIHARA K., IGUCHI K., FUKATA S., TANAKA M., YAMAMOTO Y. and MOCHIZUKI T. (1984) Immunochemical study on PHI/PHM with use of synthetic peptides. *Peptides* **5**, 247–254