Response of Plasma Prorenin and Active Renin to Chronic and Acute Alterations of Renin Secretion in Normal Humans

Studies Using a Direct Immunoradiometric Assay

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Abstract

We employed a novel immunoradiometric assay to measure plasma levels of active renin and prorenin in physiologic and pharmacologic studies designed to characterize renin biosynthesis and processing in response to both chronic and acute stimuli of renin secretion in normal human subjects. Stimulation of renin secretion with prolonged dietary sodium restriction or amiloride resulted in marked increases in the plasma levels of prorenin, active renin, and plasma renin activity (PRA); suppression of renin secretion with indomethacin resulted in parallel decreases in prorenin, active renin, and PRA. In contrast, acute stimulation with upright activity or administration of an angiotensin-converting enzyme inhibitor, which increased active renin and PRA from 2- to 15-fold, had no effect on prorenin levels. Based on studies in cultured human juxtaglomerular tumor cells, it has been proposed that prorenin is secreted constitutively whereas active renin is stored in and released from secretory granules through a regulated pathway. Our studies are consistent with such a model: the parallel changes in active renin and prorenin with experimental maneuvers of long duration suggest that both the constitutive and regulated pathways are altered under these conditions. The increase in active renin levels in the absence of a change in prorenin that occurs in response to acute stimuli presumably represents the release of preformed active enzyme that is stored in secretory granules.

Introduction

Like many polypeptide hormones and zymogens, renin (EC 3.4.99.19) is synthesized in a prepro form (1) which undergoes sequential processing to a prozymogen (prorenin) and then to the active form of the enzyme (2, 3). An increasing body of evidence indicates that the inactive form of the enzyme that accounts for 50–90% of the total amount of renin present normally in the circulation (4) is identical to prorenin (5, 6). It has been speculated that the presence of the prosegment limits access to the catalytic site which is buried in a cleft, rendering this molecule enzymatically inactive (7).

Although many investigators have reported changes in the levels of prorenin in response to a variety of physiologic and pharmacologic maneuvers in both normal subjects and patients with disorders affecting the renin–angiotensin system (4, 8, 9), a unified view of the relationship of the prozymogen to active renin has not yet emerged. To date, studies of the physiologic regulation of renin biosynthesis and processing in vivo have employed in vitro activation techniques and enzymatic assays in which plasma renin levels are quantitated by measurement of the rate at which angiotensin is released from renin substrate (angiotensinogen). Although the concentration of angiotensinogen in such assays is presumed not to be rate limiting at low enzyme concentrations (10), substrate excess is not assured at high levels of enzyme activity (11). This problem can be circumvented to some extent by addition of exogenous angiotensinogen but, even in the presence of large concentrations, a kinetic reaction that is zero order with respect to substrate is not assured. The availability of monoclonal antibodies to distinct epitopes on the human renin molecule (12, 13) has enabled us to develop immunoradiometric assays (IRMA)[1] in which the concentration of active renin and prorenin can be determined independent of the concentration of angiotensinogen. The present study reports, for the first time, the use of such direct assays to measure plasma levels of active renin and prorenin in physiologic and pharmacologic studies designed to characterize renin biosynthesis and processing in response to both chronic and acute stimuli of renin secretion in normal human subjects.

Methods

Materials

The monoclonal antibodies that were utilized in the IRMAs were prepared in high responder Biozzi mice injected with active human renin purified from a juxtaglomerular cell tumor (13, 14). The techniques for spleen cell fusion, selection of hybridomas, mass production, purification of the immunoglobulins, and characterization of the antibodies have been reported previously in detail (13). Inasmuch as the immunogen used was active renin, which lacks the prosegment portion of the parent molecule, the antibodies produced would be expected to bind to epitopes common to active renin and prorenin and not to sites present on the prosegment.

The pair of antibodies selected for the IRMA for active renin was 3E8 and 4G1 (15), and the pair for the IRMA for the direct measure-

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1. *Abbreviations used in this paper:* IRMA, immunoradiometric assay; PRA, plasma renin activity.

ment of total renin was 4E1 and 3E8. Antibodies 3E8 and 4E1 bind to different epitopes common to both active renin and prorenin and do not inhibit the enzymatic activity of human renal or plasma renin: these antibodies were selected to be the antibodies immobilized on the solid phase to trap both active renin and prorenin from plasma. Antibody 4G1 was demonstrated to be a noncompetitive inhibitor of angiotensin generation in vitro (K_i 2.5–4 × 10^{-10} mol/liter) (13) and thus is presumed to bind to an epitope on active renin near the catalytic site. Antibody 4G1 does not bind to prorenin purified from human kidney or chorionic fluid (13), to prorenin produced by chorionic cells (13) or immortalized juxtaglomerular tumor cells in culture (16), to six distinct segments of the profragment synthesized by classical techniques (17), or to other related proteins such as pepsin and cathepsin D (13). Thus, the presence of the prosegment apparently prevents access of this antibody to the epitope to which it binds.

Accordingly, antibody 4G1 was iodinated and used as the antibody specific for active renin; antibody 3E8 was iodinated and used as the second antibody in the direct assay for total renin. Antibodies were iodinated by the chloramine-T method (18). For each iodination, 1 mCi [^{125}I]sodium iodide was allowed to react with 40 μg of antibody. Free iodine was separated from the iodinated antibody on a Sephacryl S-300 column (Pharmacia, Uppsala, Sweden). The specific activity of the iodinated antibody was ~ 4 Ci/μg.

For the measurement of plasma renin concentration by enzymatic assay, angiotensinogen was prepared from nephrectomized sheep by the method of Skinner (19). ^{125}I-Angiotensin I was prepared as described by Stockigt et al. (10). Trypsin (EC 3.4.4.4) (13,900 α-*N*-benzoyl-L-arginine ethyl ester U/mg protein), soy bean trypsin inhibitor (10,000 α-*N*-benzoyl-L-arginine ethyl ester U/mg), 2,3-dimercaptopropanol (British Anti-Lewisite), and 8-hydroxy-1-azanaphthalene (8-hydroxyquinoline) were purchased from Sigma Chemical Co. (St. Louis, MO), EDTA from J. T. Baker Chemical Co. (Phillipsburg, NJ), and cyanogen bromide-activated Sepharose 4B from Pharmacia.

Subjects

Studies of the regulation of active renin and prorenin secretion were performed in 32 normal human volunteers who ranged in age from 21 to 56 yr. Only male subjects were used to avoid the potential confounding effects of variation in prorenin levels throughout the menstrual cycle (20). Informed consent was obtained from all subjects after the protocols were approved by the Committee on Human Research, University of California, San Francisco.

Subjects were hospitalized in the General Clinical Research Center at San Francisco General Hospital Medical Center where they were fed a constant metabolic diet, the composition of which is indicated in the descriptions of the individual protocols that follow. In each protocol an equilibration period, during which steady-state values for plasma and urine electrolyte composition were obtained, preceded the experimental periods which were of 4–7 d in duration. In protocols designed to test the response of renin secretion to chronic dietary or pharmacologic interventions, samples for plasma renin measurements were obtained at the end of these experimental periods or after a subsequent 6–12-d period of drug administration. Thus the measurements of renin levels obtained in these protocols reflect an experimental maneuver of relatively long (1–3 wk) duration. In protocols designed to test the response of renin secretion to acute interventions, samples for plasma renin measurements were obtained within a 3-h period after the initiation of the stimulus and thus reflect an experimental maneuver of relatively short duration.

Experimental protocols of long duration

Variation in dietary sodium intake. 29 subjects were studied after they had equilibrated on either a normal (n = 23) or low (n = 9) dietary sodium intake. Within each dietary group the quantity of sodium was adjusted on the basis of ideal weight (Metropolitan Life Insurance Tables, 1983) to provide either 1.8 mmol/kg body weight per d or < 0.1 mmol/kg per d. Dietary potassium intake (1.4 mmol/kg per d) was the same on both diets. Samples for plasma renin measurements were obtained at the end of the period of equilibration. Unless indicated otherwise, in this and in subsequent protocols, venous blood samples for measurement of plasma renin and electrolyte concentrations were obtained between 0800 and 1000 h in the fasting state after the subjects had remained recumbent overnight. Samples were collected in prechilled tubes and the plasma was separated promptly in a refrigerated centrifuge and stored frozen at −20°C until the assays were performed.

Administration of amiloride. The effect of administration of amiloride was evaluated in paired studies conducted in five subjects. During the steady-state control period the subjects ingested a constant diet containing 1.8 mmol of sodium and 1.2 mmol of potassium/kg body weight per d. During the treatment period (6–12 d) amiloride, 30–40 mg/d in four divided doses, was added to this regimen. Samples for plasma renin measurements were obtained at the completion of the control and treatment periods.

Administration of indomethacin. The effect of treatment with indomethacin was evaluated in paired studies conducted in seven subjects. During the steady-state control period the subjects ingested a constant diet containing 1.8 mmol of sodium and 1.2 mmol of potassium/kg body weight per d. During the treatment period (6–11 d) indomethacin, 150 mg daily in three divided doses, was added to this regimen. Samples for plasma renin measurements were obtained at the completion of the control and treatment periods.

Experimental protocols of short duration

Postural stimulation. The response to a change from recumbent to upright posture was evaluated in the subjects that had equilibrated on either a normal (n = 23) or low (n = 9) dietary sodium intake (*vide supra*). Plasma samples were obtained after overnight recumbency and after 2 h of upright activity. The subjects remained fasting during this interval.

Inhibition of angiotensin-converting enzyme activity. The effect of the acute administration of an angiotensin converting enzyme inhibitor on plasma levels of active renin and prorenin was evaluated in five normal subjects. To augment the stimulation of renin secretion in response to this stimulus, the subjects were studied after they had equilibrated on a low sodium intake, containing 0.2–0.3 mmol of sodium/kg body weight per d. Daily potassium intake was 0.9–1.1 mmol/kg body weight. Studies were begun between 0800 and 0900 h after the subjects had remained recumbent overnight. After placement of an indwelling intravenous catheter, blood samples were obtained before and every 30 min for 3 h after administration of captopril, 0.9 mg/kg body weight, orally. Subjects remained recumbent and fasting throughout the study. Blood pressure was monitored throughout the procedure using a semiautomatic blood pressure recording device (Dinamap model 845 XT, Critikon, Tampa, FL).

Laboratory methods

Immunoradiometric assays. All samples were assayed in duplicate. In the assay for active renin the incubation mixture included: 250-μl aliquots of plasma or renin standard (calibrated against MRC standard); 250 μl of 0.2 M imidazole buffer, pH 7.4, containing a suspension of 20 mg of Magnogel, coupled covalently to monoclonal antibody 3E8 (ERIA Diagnostics, Pasteur, Marnes La Coquette, France); and 100 μl of ^{125}I-labeled monoclonal antibody 4G1 (15). All tubes were incubated for 4 h at room temperature and shaken at a speed sufficient to maintain the Magnogel in suspension. At the end of the incubation, the solid phase was retained in the tube with a magnetic bar, and the supernatants were discarded. The active renin, which is sandwiched between the two antibodies, was quantified by counting the amount of bound ^{125}I.

Fig. 1 illustrates a typical assay curve obtained using serial dilutions of standard active renin. A parallel displacement of ^{125}I-labeled antibody 4G1 was observed with serial dilutions of active renin produced by trypsinization of the supernatant of human chorionic cells in culture (21). In contrast, minimal activity was detected with the addition of nonactivated prorenin from the same culture medium. This cross-

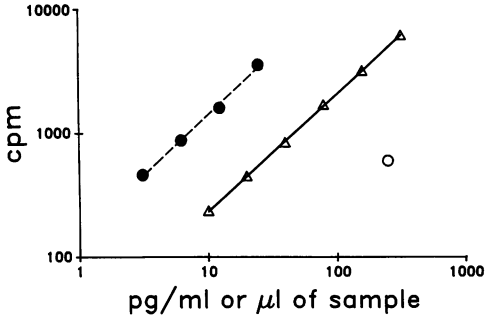

Figure 1. Immunoradiometric assay of human renin using monoclonal antibodies 3E8 and 4G1. (△ — △) Standard curve, plotted in picograms per milliliter, obtained using the Medical Research Council human renin standard (World Health Organization preparation 68/356). (● --- ●) Parallel displacement of ^{125}I-labeled antibody 4G1 observed with serial dilutions of active renin, plotted in microliters of sample, produced by trypsinization of the supernatant of human chorionic cells in culture (21), using the method of Sealey et al. (22). (○) Displacement obtained with an undiluted sample of the same supernatant before trypsinization, which contains predominantly prorenin. As is evident from the figure the cross-reactivity of this untrypsinized supernatant is < 1.5% in the assay. This cross-reactivity probably represents active renin present in the untreated supernatant, inasmuch as incubation of this material with human angiotensinogen indicated that an equivalent amount of enzymatically active renin was present.

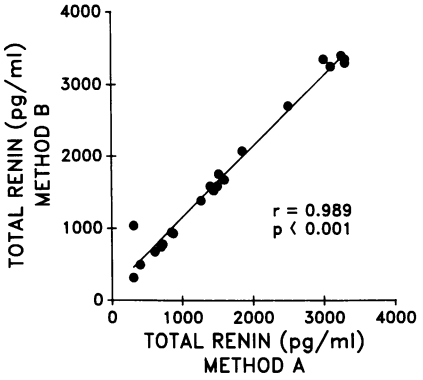

Figure 2. Comparison of plasma total renin concentration measured by two different methods. In method *A* total renin (prorenin plus active renin) is measured using a pair of monoclonal antibodies (3E8 and 4E1) that bind to different epitopes common to both molecular species of renin. In method *B* total renin is measured as the amount of active renin present after activation of prorenin with trypsin; active renin is measured using a pair of monoclonal antibodies (3E8 and 4G1) that permit specific detection of an epitope unique to active renin. A highly significant correlation between results obtained using these two different methods was evident over a wide range of total renin values.

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In the method described active renin levels of > 5 pg/ml can be detected. Assay sensitivity can be increased by a prolonged incubation step before the addition of the labeled second antibody but this step was not required for the range of renin values encountered in the present studies. The intraassay and interassay coefficients of variation are 6% and 8%, respectively, for active renin concentrations in the range of 60–65 pg/ml.

Total renin was measured by two independent techniques. In the direct assay for total renin (method A) the incubation mixture included: 250 μl of plasma or renin standard; 250 μl of the suspension of Magnogel coupled covalently to antibody 4E1; and 100 μl of ^{125}I-labeled antibody 3E8. The conditions of this assay were otherwise identical to those utilized for the direct assay of active renin. In the second method (method B) prorenin was activated using the method of Sealey et al. (22), in which trypsin is incubated with plasma at a concentration of 1 mg/ml for 1 h at −4°C. After the renin was activated total renin was measured using the IRMA for active renin described above. Nearly identical results were obtained when total renin levels were measured with these two different IRMA techniques using separate aliquots of plasma samples that encompass a wide range of renin levels (Fig. 2). In both methods, prorenin is calculated as the difference between the values of total renin and active renin.

Enzymatic assays. In the protocol in which renin secretion was stimulated by administration of captopril, active and total renin levels were also measured enzymatically as the rate of angiotensin I generation in the presence of heterologous angiotensinogen before and after activation. Activation was accomplished using the method of Derkx et al. (23), in which trypsin, bound covalently to Sepharose, is incubated with plasma at a concentration of 0.25 mg/ml for 24 h at +4°C. In preliminary studies we found that total renin concentrations achieved by this technique were highly correlated with total renin values after acid activation using the method of Day and Luetscher (24): total renin (trypsin) = 0.96 × total renin (acid) + 3.4, $r = 0.98$, $P < 0.001$, $n = 34$, for samples ranging from 20 to 420 ng/ml per h. In this assay, plasma was incubated at 37°C with 450 μl of concentrated angiotensinogen. The incubation was carried out in a final volume of 1.0 ml in the presence of 15 mM EDTA, 5 mM British Anti-Lewisite, and 3.5 mM 8-hydroxyquinoline at a final angiotensinogen concentration of ~ 1 μM.

It was evident in initial studies employing a 2 h incubation that the volume of plasma used routinely for measurement of plasma renin concentration contained so much active enzyme under these study conditions that the enzyme reaction was not proceeding at a constant rate with time. In an attempt to optimize the kinetics of this reaction, the incubation time was limited to 30 min and the volume of plasma was adjusted so that the rate of angiotensin generation was comparable between samples and did not exceed 10 ng per 30 min in any incubation tube. Accordingly, the concentration of renin in the plasma samples is expressed as the rate of angiotensin I generated during the 30-min incubation period after correction for the amount of preformed angiotensin I present in unincubated plasma. Under these study conditions, the results of both active renin and total renin measurements obtained by the enzymatic assay were highly correlated with those obtained by the IRMA on the same plasma samples (Fig. 3). As in the IRMAs, prorenin was calculated as the difference between total and active renin levels.

Plasma renin activity (PRA) was measured by radioimmunoassay of the angiotensin I generated in the presence of endogenous angiotensinogen. The assay conditions have been described previously in detail (25).

Miscellaneous methods. Plasma and urine sodium and potassium concentrations were measured by flame photometry. Urinary excretion of aldosterone-18-glucuronide was measured by radioimmunoassay (26).

All data are summarized as the mean±SEM for the sample studied. Pairs of means from independent samples were compared using Student's *t* test for unpaired data (27). Nonparametric tests of comparison (Wilcoxon's signed-ranks test, Dunnett's test for multiple comparison's with a control) were used when the sample size was small (27, 28).

Results

Protocols of long duration. Plasma concentrations of active renin, total renin, and prorenin as well as PRA varied inversely

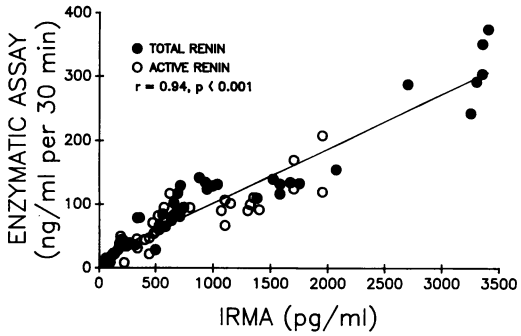

Figure 3. Comparison of renin measurements obtained by enzymatic and immunoradiometric assays on 45 plasma samples obtained from normal subjects. Samples were selected to encompass a wide range of renin levels obtained during various physiologic and pharmacologic maneuvers. Active renin was measured on these samples by the standard enzymatic assay and by the IRMA employing monoclonal antibodies 3E8 and 4G1. Total renin was measured using the same techniques following activation by exposure to trypsin using the method of Derkx et al. (23). A highly significant correlation between results obtained using these two methods was evident for all samples as well as when the samples for active renin ($r = 0.90$, $p < 0.001$) and total renin ($r = 0.96$, $p < 0.001$) were considered separately. Similarly, an excellent correlation between the IRMA for active renin and measurements of plasma renin activity (PRA = 0.11 (IRMA) − 0.7, $r = 0.902$, $p < 0.001$) was obtained in a separate group of 98 plasma samples obtained in 49 normal subjects ranging in age from 20 to 65 yr.

with dietary sodium intake (Table I). In comparison to levels obtained in subjects ingesting a normal sodium diet, values of both active renin and PRA were six- to sevenfold higher in those ingesting the lower sodium intake.[2] Although the total renin and prorenin levels were also greater in the sodium restricted group, the percentage of the total renin present in the active form was greater in the subjects ingesting the low sodium intake. Plasma sodium concentration and urinary sodium excretion were lower and aldosterone excretion was greater in the subjects who ingested the lower sodium intake. Plasma potassium concentration and urinary potassium excretion did not differ significantly between the two dietary groups. Plasma concentrations of active renin, total renin, prorenin, and PRA also increased in response to administration of amiloride (Table II, Fig. 4). In comparison to pretreatment levels, values of both active renin and PRA increased 7–33-fold. Although total renin and prorenin levels also increased substantially, the percentage of the total renin present in the active form increased significantly to an average value of 64%. At the doses employed in these studies, treatment with amiloride resulted in a marked natriuresis (cumulative increase in urinary sodium excretion 458±27 mmol, $P = 0.04$),[3] weight loss (1.9±0.5 kg, $P = 0.04$), and a significant decrease in plasma sodium levels (Table II). Potassium retention (cumulative decrease in urinary potassium excretion 65±29 mmol, $P = 0.13$), and a significant increase in plasma potassium concentration also occurred. Urinary aldosterone excretion increased nearly 20-fold, reflecting the concomitant increases in potassium and renin levels.

Administration of indomethacin resulted in a significant decrease in plasma concentrations of active renin, total renin, prorenin, and PRA (Table II, Fig. 5). In comparison to pretreatment values both active renin and PRA decreased by 33–95%. Although total renin and prorenin levels also decreased substantially, the percentage of total renin present in the active form decreased to an average value of 13%. Urinary aldosterone excretion decreased significantly during the period of indomethacin treatment.

Protocols of short duration. In response to assumption of the upright posture, plasma concentrations of active renin, total renin, and PRA increased significantly in subjects ingesting both a normal and a low sodium diet (Table III). Prorenin levels did not change significantly in either group. The percentage of total renin present in the active form increased significantly in both groups of subjects.

The response of plasma renin concentrations to administration of captopril is shown in Fig. 6. In both the immunoradiometric (*upper*) and enzymatic (*lower*) assays, active renin levels increased by more than sixfold in each subject (mean 10.7±1.4-fold for the IRMA and 11.6±3.1-fold for the enzymatic assay). Peak values of active renin ranged from 700 to 1,950 pg/ml and from 60 to 208 ng/ml per 30 min, respectively. In contrast, plasma concentrations of prorenin did not change significantly in response to captopril administration, whether measured enzymatically or by the IRMA. Mean arterial blood pressure decreased in each subject with an average decrease of 17±7 mmHg for the group as a whole. Plasma sodium and potassium concentrations did not change significantly during the study.

Discussion

The results of the present studies, utilizing direct IRMAs that distinguish between and quantitate active renin and prorenin, indicate that the concentrations of both of these forms of renin in human plasma vary, to different extents, in response to physiologic and pharmacologic maneuvers designed to stimulate or suppress renin secretion. The principal determinant of the pattern of this response is the duration of the experimental period. Whereas parallel changes in active renin and prorenin levels occurred in each of the protocols that were conducted over a period of days to weeks (variation in dietary sodium

2. Results obtained from this assay, which are expressed in picograms per milliliter, can be compared to those expressed in IU using the Medical Research Council standard (World Health Organization international reference preparation 68/356). The conversion factor of 1 pg = 1 IU is based on the specific activity of the purified renin preparation which is in the range of 800–1,000 IU/mg of protein. Using this conversion factor our average values for active renin during ingestion of normal salt and low salt diets of 45 and 328 pg/ml, respectively, are quite similar to the levels of 56 and 221 IU reported to be present in plasma obtained under similar study conditions by Bangham et al. (29).

3. Cumulative changes in sodium and potassium excretion were calculated as the sum of the daily differences between the excretion of these ions during amiloride treatment and the mean excretion rate for the respective ion during the steady-state control period that preceded amiloride treatment.

*Table I. Effect of Variation in Dietary Sodium Intake on Plasma Renin Concentrations, Plasma and Urine Electrolytes, and Urine Aldosterone Excretion in Normal Control Subjects**

Intake								Plasma		Urine		
Na	K	No. of subjects	PRA	Active renin	Total renin	Prorenin	Active/ total	Na	K	Na	K	Aldosterone
mmol/24 h			*ng/ml/h*		*pg/ml*		%	*mmol/liter*		*mmol/24 h*		*μg/24 h*
126±3	90±3	23	2.2±0.3	45±6	218±39	173±37	25±2	141±1	4.0±0.1	106.5±3.4	75.7±2.9	14.8±1.6
4±1	97±3	9	12.6±1.9	328±64	977±176	649±122	35±2	137±1	4.1±0.1	1.3±0.3	83.8±2.8	93.0±10.8
Intergroup comparisons: *P*												
<0.001	NS		<0.001	<0.001	<0.001	<0.001	<0.05	<0.001	NS	<0.001	NS	<0.001

* Values are the mean±SEM of the average values obtained during a 4–7-d steady-state observation period in each subject. Comparison between groups was done using Student's *t* test for unpaired variables. NS, nonsignificant ($P > 0.05$).

intake, treatment with amiloride or indomethacin), only active renin levels changed in protocols that took place over 2–3 h (upright activity or administration of an angiotensin-converting enzyme inhibitor). In these latter protocols, a marked dissociation in the response of these two molecular forms of renin was evident inasmuch as prorenin levels did not change significantly despite an increase in active renin levels and PRA of up to 15-fold.

Prorenin is generally considered to be the predominant form of renin in human plasma (8, 9, 30). It is apparent from our results that, whereas prorenin represents more than half of the total renin under most physiologic conditions, the percentage of the total renin present in the plasma in the proform varies inversely with the absolute levels of active renin (Fig. 7). Thus, under conditions in which renin secretion is markedly stimulated, the active form may predominate: prorenin comprises an average of only 31–36% of the total plasma renin when active renin levels are markedly increased with amiloride or angiotensin-converting enzyme inhibitors. In contrast, prorenin comprises nearly 90% of the total renin in subjects in whom active renin levels are suppressed by indomethacin. That the percentage of total renin in the proform varies inversely with the level of active renin was also evident in the studies of Atlas et al. (31) in which a variety of pharmacologic agents were used to stimulate and suppress renin secretion.

Prorenin, like active renin, is synthesized in and released by the juxtaglomerular cells in the kidney (32). Prorenin has also been identified in other tissues (33–35), perhaps accounting for the persistence of this prozymogen in plasma after bilateral nephrectomy (36–38). A small quantity of the precursor of other polypeptide hormones (e.g., proinsulin) escapes intracellular processing and is secreted into the circulation (39); no physiologic role has been defined for these circulating peptides. In contrast, because prorenin is usually present in the circulation at levels greater than those of active renin, it has been suggested that such large quantities of the prozymogen may represent a reserve form of the enzyme (40). The observation that active renin is virtually undetectable in the plasma

*Table II. Effect of Treatment with Amiloride or Indomethacin on Plasma Renin Concentrations, Plasma and Urine Electrolytes, and Aldosterone Excretion in Normal Control Subjects**

Study condition	PRA	Active renin	Total renin	Prorenin	Active/ total	Plasma		Urine		
						Na	K	Na	K	Aldosterone
	ng/ml/h		*pg/ml*		%	*mmol/liter*		*mmol/24 h*		*μg/24 h*
Amiloride treatment, $n = 5$										
Control	3.2±0.7	63±12	200±28	137±19	31±3	139±0.6	4.2±0.1	105±7	69±3	12.2±2.4
Treatment	36.9±5.0	1396±364	2070±442	674±111	64±7	131±1.6	5.5±0.2	124±7	74±6	232.7±59.8
P	=0.04	=0.04	=0.04	=0.04	=0.04	=0.04	=0.04	=0.04	NS	=0.04
Indomethacin treatment, $n = 7$										
Control	2.5±0.4	43±10	201±88	158±80	29±5	142±1	4.0±0.1	121±2	81±5	19.6±4.0
Treatment	0.4±0.1	8±2	62±12	54±10	13±3	142±1	4.2±0.1	124±6	67±3	4.8±1.4
P	=0.02	=0.02	=0.02	=0.02	=0.09	NS	=0.04	NS	=0.03	=0.02

* Values for plasma renin concentrations are the mean±SEM of the measurements obtained on each subject at the end of the steady-state control and treatment periods. Values for plasma and urine electrolytes and aldosterone excretion are the mean±SEM of the average of multiple measurements in each subject during the experiment periods. The means were compared using Wilcoxon's signed-ranks test for paired variables. NS, nonsignificant ($P \geq 0.1$).

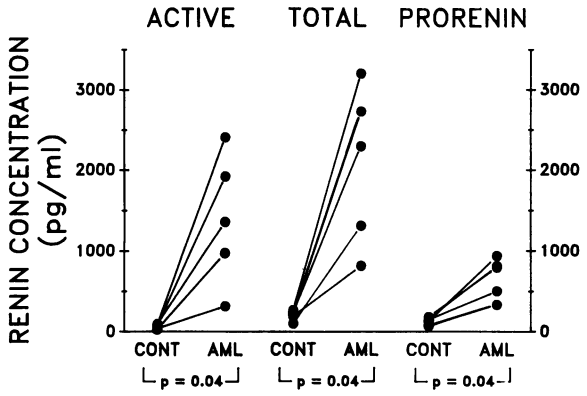

Figure 4. Response of plasma active renin, total renin, and prorenin levels to administration of amiloride in five normal control subjects. Plasma samples were obtained after overnight recumbency after a steady-state control period (*CONT*) and at the completion of a subsequent 6–12-d experimental period during which amiloride (*AML*) was administered at a dose of 30–40 mg/d. Data from the *CONT* and *AML* treatment periods were compared using Wilcoxon's signed-ranks test.

of anephric patients (36–38), however, provides evidence against the notion that significant activation of prorenin normally occurs in the peripheral plasma. This observation does not preclude the possibility that prorenin itself may exert a physiologic effect: roles as potential cardiotropic (35) and reproductive (41) hormones have been suggested.

Based on their studies utilizing cultured cells derived from a human renin-producing tumor, and on the general model for protein secretion suggested by Kelly (42), Galen et al. (3) have proposed a two-pathway model of renin biosynthesis, processing, and secretion. In this model, preprorenin is synthesized in the rough endoplasmic reticulum where the prefragment is rapidly removed and the resultant prorenin is transported to the Golgi apparatus. The molecule then reaches the circulation

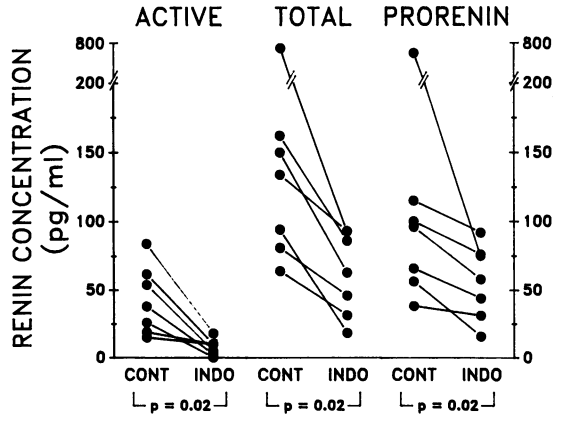

Figure 5. Response of plasma active renin, total renin, and prorenin levels to administration of indomethacin in seven normal control subjects. Plasma samples were obtained following overnight recumbency after a steady-state control period (*CONT*) and at the completion of a subsequent 6–11-d experimental period during which indomethacin (*INDO*) was administered at a dose of 150 mg/d. Data from the *CONT* and *INDO* treatment periods were compared using Wilcoxon's signed-ranks test.

by one of two pathways: in the first or regulated pathway, prorenin is processed and stored in secretory granules from which active renin is released into the plasma by exocytosis in response to acute stimulation; in the second pathway, unprocessed prorenin is secreted constitutively without formation of secretory granules. The results of the present studies conducted in normal human subjects are consistent with this two-pathway model. Acute stimulation of renin secretion resulted in an increase in the plasma concentration of active renin in the absence of a detectable change in the level of prorenin suggesting that under these conditions the secreted renin originated as preformed active enzyme stored in secretory granules (Fig. 4). Chronic stimulation of renin secretion caused a parallel change in the release of both prorenin and active renin (Fig. 7), presumably reflecting an overall increase in the rate of hormone biosynthesis and augmented secretion by both the constitutive and regulated pathways. Since the percentage of the total renin in the active form also increased with chronic stimuli and decreased with chronic suppression, it appears that, under such conditions, there is intracellular regulation of the proportion of precursor that is processed to the biologically active enzyme.

The conclusions drawn from our studies in which renin levels were measured as the concentration of the molecular species in plasma accord in many but not all respects with those drawn from previous studies in which changes in prorenin and active renin were assessed by enzymatic assays. In agreement with our results, plasma prorenin levels increased in experiments in which active renin levels were stimulated chronically by dietary sodium restriction, diuretic therapy, or treatment with inhibitors of angiotensin converting enzyme (30, 38, 43–46). In contrast to our results, prorenin levels did not decrease in a previous report in which active renin levels were suppressed by indomethacin (47). Perhaps our observation that such treatment decreased plasma prorenin levels (Table II, Fig. 5) was due to the longer period of treatment in our study (6–11 d vs 3 d).

Even more significant differences between the results obtained using IRMA and enzymatic techniques occurred in studies employing physiologic or pharmacologic maneuvers that caused an acute stimulation of renin release. In agreement with our results using the IRMA technique, several studies employing enzymatic assays have found that acute stimulation of active renin levels by assumption of the upright posture or acute administration of furosemide, saralasin or captopril were not accompanied by significant changes in prorenin levels (43, 45, 47). However, when an angiotensin-converting enzyme inhibitor was administered to normal subjects ingesting a low sodium diet, the marked increases in active renin observed (averaging 12-fold) were reportedly accompanied by concomitant decreases in prorenin to less than 10% of control levels within the following 3 h (46). Using similar study conditions, we also noted a 12-fold increase in active renin, yet plasma prorenin levels remained unchanged (Fig. 6, *top*). It seems unlikely that the differences between these two studies could be explained by any inherent difference between IRMA and enzymatic methods since we obtained qualitatively similar results when aliquots of the same plasma samples were assayed by enzymatic techniques (Fig. 6, *bottom*).

Although the qualitative results obtained using the IRMA and enzymatic methods in our study were similar, it is possible

*Table III. Effect of a Change from Recumbent to Upright Posture on Plasma Renin Concentrations in Normal Control Subjects Ingesting a Normal or Restricted Sodium Intake**

Study condition	No. of subjects	PRA		Active renin		Total renin		Prorenin		Active/total	
		Rec	Up	Rec	Up	Rec	Up	Rec	Up	Rec	Up
		ng/ml/h		*pg/ml*		*pg/ml*		*pg/ml*		%	
Normal sodium diet	23	2.2±0.3	4.9±0.8	45±6	91±14	218±39	263±38	173±37	171±36	25±3	37±4
Restricted sodium diet	9	12.6±1.9	20.7±3.1‖	328±64	519±85§	977±176	1144±191‡	649±88	626±126	35±2	47±4

* Values are the mean±SEM. Comparisons between recumbent (Rec) and upright (Up) samples were made using Student's *t* test for paired variables: ‡ $P = 0.02$, § $P = 0.008$, ‖ $P = 0.003$, $P < 0.001$.

that different results would have been obtained if the conditions of the enzymatic reaction had been less rigorous. The amount of available angiotensinogen in the in vitro assay of renin influences the quantity of angiotensin I which is generated. If not enough substrate is available, the production of angiotensin I after trypsin activation of renin is underestimated and the measurement of prorenin, based on the difference between the amount of angiotensin I generated by native plasma and trypsin-treated plasma, might be grossly underestimated. This could result in an apparent decrease in circulating prorenin that is a consequence of the inappropriate renin–angiotensinogen kinetics in vitro, and not a physiologically relevant finding.

These considerations emphasize the advantage of a method which is independent of the kinetics of the enzymatic reaction and which provides an estimate of the actual concentration of the different molecular forms of renin present in plasma. However this methodology is still limited since it does not provide a direct measure of prorenin in plasma, but rather only a calculated value derived by subtracting the concentration of active renin from that of total renin (48). Further, it remains to be determined whether these antibodies will distinguish an enzymatically active form of prorenin such as has been demonstrated to occur following acid activation in vitro (49), al-

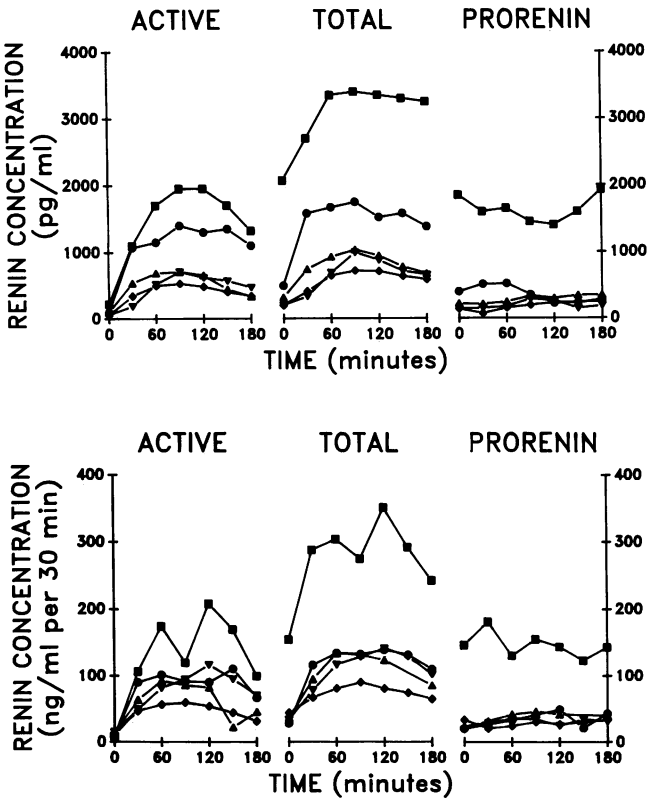

Figure 6. Response of plasma active renin, total renin, and prorenin levels to administration of captopril in five normal control subjects. Plasma samples were obtained before and every 30 min during a 3-h period after the oral administration of captopril, 0.9 mg/kg body weight. The subjects, who are distinguished by the different symbols, were studied after they had equilibrated on a low sodium diet (containing 0.2–0.3 mmol sodium/kg body weight per d). The subjects had fasted and remained overnight and remained so during the study. In response to captopril, plasma levels of both active and total renin increased whereas those of prorenin did not. Comparable results were obtained when separate aliquots of each plasma sample were analyzed by either the IRMA (*top*) or enzymatic (*bottom*) methodologies.

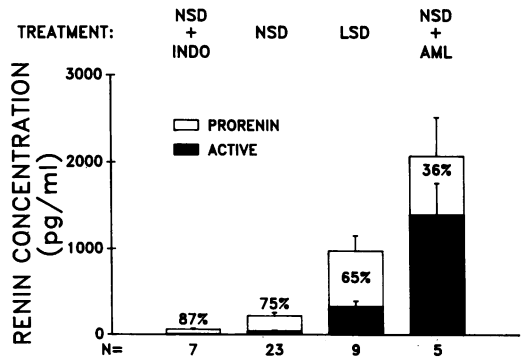

Figure 7. Comparison of the plasma levels of active renin and prorenin in normal subjects during various protocols designed to chronically increase or decrease renin secretion. Samples were obtained after overnight recumbency after the subjects had equilibrated on each of the experimental protocols: *NSD*, normal salt diet (1.8 meq/kg body weight per d); *LSD*, low salt diet (< 0.1 meq/kg per d); *INDO*, indomethacin (150 mg/d); *AML*, amiloride (30–40 mg/d). Active renin levels are shown by the filled areas, prorenin by the empty areas, and total renin by the total height of the bar. Values are the mean±SEM and the number of subjects in each protocol are shown by the number below each bar. The percentage of total renin present as prorenin is shown within (or above) the open portion of the bar.

though the existence and physiologic relevance of this so-called intermediate form of prorenin in circulating plasma has not been established.

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