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Renin and prorenin disappearance in humans post-nephrectomy: evidence for binding?

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1. ABSTRACT

To study the distribution of kidney-derived renin-angiotensin system (RAS) components in humans, we monitored the decline in plasma prorenin, renin, angiotensin (Ang) I and Ang II post-nephrectomy. Prorenin and renin decreased biphasically, prorenin displaying a slower elimination. The distribution half life was similar for both. Angiotensins followed the disappearance of renin. One-two days post-nephrectomy, stable plasma concentrations at 5-10% (renin and angiotensins) and 25-30% (prorenin) of pre-nephrectomy levels were reached. The total amount of kidney-derived renin and prorenin in the body was ≈10 times as much as the amount in blood. Prorenin also originated at extrarenal sites. The renin levels in anephrics corresponded with the percentage of prorenin that *in vitro* has a so-called 'open conformation' (i.e., displays enzymatic activity), suggesting that renin in anephrics is in fact 'open' prorenin. Haemodialysis nor captopril significantly affected the level of any RAS component in anephrics. In conclusion, renal renin/prorenin enter tissue sites in humans, and renal renin is the main determinant of plasma angiotensins. Whether prorenin contributes to tissue angiotensin generation in humans remains to be determined.

2. INTRODUCTION

Angiotensin (Ang) production occurs at tissue sites rather than in circulating blood (1,2). Such tissue production efficiently results in the activation of Ang II type 1 (AT_1) receptors and/or Ang II type 2 (AT_2) receptors, and requires the local presence of renin, angiotensinogen and ACE. Yet, although ACE is indeed expressed locally in multiple organs, renin and angiotensinogen are not (3-6). Thus, to allow Ang I synthesis at tissue sites, renin and angiotensinogen need to be taken up from circulating blood. Indeed, both proteins diffuse into the interstitial fluid (5,7,8). In addition, renin binds to receptors. Up to now, two of such receptors have been described: the mannose 6-phosphate (M6P) receptor (which is identical to the insulin-like growth factor II receptor) and the 'renin receptor'. M6P receptors bind any phosphomannosylated (M6P-containing) protein, and therefore cannot be considered as renin-specific receptors. These receptors most likely contribute to renin clearance (9-11). Renin receptors bind renin on the cell surface (12,13).

Importantly, both receptors also bind prorenin, the inactive precursor of renin (12-14). The renin receptor is

therefore currently known as the (pro)renin receptor. M6P receptor-bound prorenin is internalized, and, as part of a clearance process, is converted to renin and subsequently degraded (11,15). (Pro)renin receptor-bound prorenin remains on the cell surface, and, due to a conformational change, becomes catalytically active. Recent studies suggest that prorenin, rather than renin, is the endogenous 'agonist' of this receptor (13,16). Consequently, tissue angiotensin generation may actually depend on prorenin (17). Such a role for prorenin would explain not only why we have so much prorenin (its levels in blood are usually >10-fold higher than those of renin) (18), but also why some extrarenal organs selectively release prorenin into the circulation. Examples of prorenin-releasing organs are the ovary, the testis, the eye, and the adrenal (19-22).

Up to now, all evidence for prorenin contributing to tissue angiotensin production comes from animal studies (14,23-25). Such studies have made use of animals overexpressing prorenin or the (pro)renin receptor, and/or the application of a (pro)renin receptor antagonist, the so-called handle region peptide (HRP). Studies in humans on this topic are, for obvious reasons, much more difficult to perform, if not impossible.

In the present study, we set out to investigate the kinetics of renin and prorenin in humans, making use of subjects undergoing a complete nephrectomy, resulting in the removal of their last remaining kidney. A detailed analysis of the disappearance curves of both proteins provides insight into their tissue distribution. In addition we quantified the levels of renin-angiotensin system (RAS) components in subjects that had been anephric for several years, both before and after haemodialysis, and we studied the effect of an ACE inhibitor (captopril) on these levels.

3. METHODS

The studies were approved by the Ethics Committee of the Erasmus MC, and all subjects gave their informed consent.

3.1. Subjects undergoing nephrectomy

Four subjects (3F, 1M; age 53-69 years) undergoing nephrectomy because of chronic pyelonephritis or renal adenocarcinoma participated in the study. One subject was taking 25 mg captopril twice daily. Blood samples for RAS component measurements were taken from an antecubital vein 30 minutes before, during (t=0) and 10, 20, 30, 60, 120, 240, 480, 720, 1440 and 2880 minutes after clamping of the renal vein and subsequent removal of the kidney.

3.2. Anephric subjects

Thirteen anephric subjects (9F, 4 M; age 24-79 years), who had been anephric for 1-17 years (mean 8 years), participated in the study. The reasons for nephrectomy were chronic pyelonephritis (n=4), chronic interstitial nephritis (n=1), chronic glomerulonephritis (n=1), renal adenocarcinoma (n=2), M. Goodpasture (n=1), polycystic kidneys (n=2), reflux nephropathy (n=1), and uncontrollable severe hypertension (n=1). All subjects were receiving maintenance haemodialysis, 2-3 times a week, with a Fresenius A2008C dialyzer and a disposable polyacrylonitryl membrane kidney. The subjects were seated for 30 minutes before the haemodialysis was started and remained seated during the procedure. Blood samples for RAS component measurements were taken from an antecubital vein immediately before and after haemodialysis.

On a second occasion, 5 anephric subjects (4 F, 1M; age 38-67 years) were given 50 mg captopril orally after they had been seated for 30 minutes. Haemodialysis was started 2 hours after captopril and lasted 4 hours. Blood samples for RAS component measurements were taken from an antecubital vein immediately before and 2 hours and 6 hours after captopril was given. The patients remained seated throughout the entire period. Blood pressure was measured by standard sphygmomanometry at 0, 30, 45, 60, 90, 120 and 180 minutes after captopril had been taken. Heart rate was computed from a continuous electrocardiographic tracing.

For comparison, blood samples for renin, prorenin, Ang I and Ang II measurements were taken from 17 healthy controls (9F, 8M; age 21-60 years), after they had been seated for 30 minutes.

Blood for renin, prorenin, angiotensinogen, ACE and total protein measurements was centrifuged at 3000 g for 10 minutes at room temperature, and plasma was stored at -20°C (26). Blood for angiotensin measurements was centrifuged at 3000 g for 10 minutes at 4°C, and plasma was stored at -70°C (26).

3.3. Biochemical measurements

Renin and prorenin were measured by enzyme-kinetic assay (EKA) (27). To measure prorenin by EKA, it was first converted to renin by incubation with Sepharose-bound trypsin. Results of the enzyme-kinetic assay are expressed as mU/L using the WHO human kidney renin standard 68/356 as reference standard. The lower limit of detection was 0.5 mU/L. Renin + prorenin ('total renin') were also measured by immunoradiometric assay (IRMA), using the monoclonal antibodies R3-27-6 and R3-36-16 (28). These two antibodies recognize different epitopes of the renin molecule and react equally well with renin and prorenin. The results of this assay are also expressed as mU/L, and the lower limit of detection was 5 mU/L.

Angiotensinogen was determined as the maximum quantity of Ang I generated in the presence of excess human kidney renin (29). The lower limit of detection was 1 nmol/L. Ang I and II were measured by radioimmunoassay after SepPak extraction and high-performance liquid chromatography separation. The lower limit of detection was 0.5 pmol/L for Ang I, and 0.25 pmol/L for Ang II, respectively (2). ACE activity was measured with a commercial kit (ACEColor, Fujirebio, Tokyo, Japan). Total protein was measured with a routine laboratory method.

3.4. Statistical analysis

Data are presented as mean±SD or geometric mean and range. The plasma disappearance of RAS

Table 1. Plasma prorenin, renin, angiotensin I and angiotensin II levels (geometric mean and range) before and after nephrectomy in 4 subjects. At t=0 the renal vein was clamped.

Time (hours)	**Prorenin** (mU/L)	**Renin** (mU/L)	**Angiotensin I** (pmol/L)	**Angiotensin II** (pmol/L)
-0.5	260 (92-1471)	55.7 (26.2-160)	69.1 (16.5-215)	12.8 (2.3-93.3)
0	278 (116-1656)	57.3 (26.4-145)	70.5 (17.6-187)	14.3 (3.8-77.2)
48	59.3 (15.5-107)	1.5 (0.6-3.1)	1.9 (0.5-4.1)	1.0 (0.6-1.4)

Table 2. RAS component plasma levels (geometric mean and range) in 13 anephric subjects (Nx) before and after haemodialysis and in 17 healthy controls.

RAS component	**Nx Before**	**Nx After**	**Controls**
Prorenin (mU/L)	86.3 (25.5-537)	87.1 (20.7-676)	161 (75-548)
Renin (mU/L)	0.9 (0.3-4.4)	0.9 (0.3-4.0)	13.2 (2.7-39.1)
Angiotensinogen (nmol/L)	2736 (1700-5277)	2879 (1737-6071)	not done
ACE (U/L)	11.8 (4.0-20.3)	13.5 (4.5-21.6)	not done
Angiotensin I (pmol/L)	4.5 (1.6-6.5)	5.2 (1.8-8.6)	21 (8.0-53)
Angiotensin II (pmol/L)	1.1 (0.4-2.8)	1.0 (0.3-4.3)	4.0 (1.2-8.5)

components after nephrectomy was studied by plotting the plasma levels semilogarithmically against time. The plasma levels reached 24-48 hours after nephrectomy were similar to those found in subjects who had been nephrectomized for several years, and these levels were therefore considered to be of non-renal origin. In order to correct for RAS components of non-renal origin, the plasma levels found 48 hours after nephrectomy were subtracted from the actual levels as measured during the first 12 hours. The plasma disappearance curves were then analyzed using a two-compartment exponential model. Differences between half lives were evaluated using Scheffé's test for multiple comparison. Associations were assessed by calculating Pearson's coefficient of correlation. Differences between plasma levels before and after haemodialysis or captopril were evaluated using Student's t-test for paired observations. Values were considered significant at $P<0.05$.

4. RESULTS

4.1. Subjects undergoing nephrectomy

RAS component levels at 0.5 hour prior to the clamping of the renal vein and the moment of clamping (t=0) were identical (Table 1). The plasma levels of prorenin, renin, Ang I and Ang II dropped rapidly after clamping of the renal vein and subsequent removal of the kidney (Figure 1 and Table 1). Prorenin levels reached a plateau at ≈25-30% of the levels at t=0, whereas renin, Ang I and Ang II decreased to levels <5% of the levels at t=0. Ang I and II were still above the detection limit after 48 hours (Figure 2 and Table 1). The disappearance curves were biphasic, and the half life of the fast component of the curve (1.0±0.2, 0.7±0.2, 0.6±0.2 and 0.7±0.3 hour for prorenin, renin, Ang I and Ang II, respectively) was identical for all 4 RAS components. The half life of the slow component (which corresponds with the effective plasma half life) was significantly ($P<0.05$) longer for prorenin (7.6±2.0 hours) than for the other 3 RAS components (2.8±0.6, 3.3±0.4 and 3.5±0.7 hours for renin, Ang I and Ang II, respectively). The area under the curve (AUC) for prorenin was 234 (geometric mean; range 61.6-1299) U/L.min, as opposed to 17.7 (10.4-45.0) U/L.min for kidney-derived renin.

4.2. Anephric subjects

Haemodialysis did not alter the levels of renin, prorenin, angiotensinogen, ACE, Ang I and Ang II (Table 2). EKA measurements were identical to IRMA measurements (Figure 3), confirming that the Ang I-generating activity measured in the EKA was entirely renin-dependent. The renin, Ang I and Ang II levels in anephric subjects were ≈5-10 times lower than those in healthy controls, whereas the prorenin levels in anephric subjects were ≈2 times lower than in healthy controls (Table 2). In anephric subjects, renin amounted to 2.0±0.8% of total renin, and correlated strongly with prorenin (Figure 4; $r=0.97$, $P<0.001$). In healthy controls, renin amounted to 8.4±4.0% of total renin ($P<0.01$ vs. anephric subjects), and.correlated less well with prorenin (Figure 4; $r=0.64$, $P<0.01$). In 6 subjects (5F, 1M), total renin had also been measured 4 years prior to this study. Results were identical to those measured here (47±13 vs. 41±6 mU/L). The relationship between renin and Ang I and the relationship between Ang I and Ang II were identical in healthy and anephric subjects (Figure 5).

Captopril reduced plasma Ang II in anephric subjects by 50-60% (P=NS) 2 hours after the dose. It did not alter the plasma levels of prorenin, renin, angiotensinogen, Ang I or total protein (Figure 6), nor did it affect blood pressure and heart rate (Figure 7).

5. DISCUSSION

This study demonstrates that in humans, kidney-derived renin and prorenin are not limited to the circulation. The distribution of both proteins over the body occurs relatively fast, with a half life of 0.5-1 hour, whereas their metabolism takes place much slower, with a half life of several hours. The latter resembles data in cynomolgus monkeys and marmosets following infusion of prorenin (30,31). The elimination half life of prorenin in humans was twice as long as that of renin. This probably relates to the larger hepatic extraction of renin versus prorenin (hepatic vein/arterial concentration ratio 0.8±0.1 vs. 0.9±0.1, n=115, $P<0.01$; F.H.M. Derkx *et al.*, unpublished observations). Differences in glycosylation most likely underlie these differences in hepatic extraction (32). Since the liver blood flow corresponds with ≈25% of cardiac output (≈3 L plasma/min), it can be calculated that the hepatic clearances of renin and

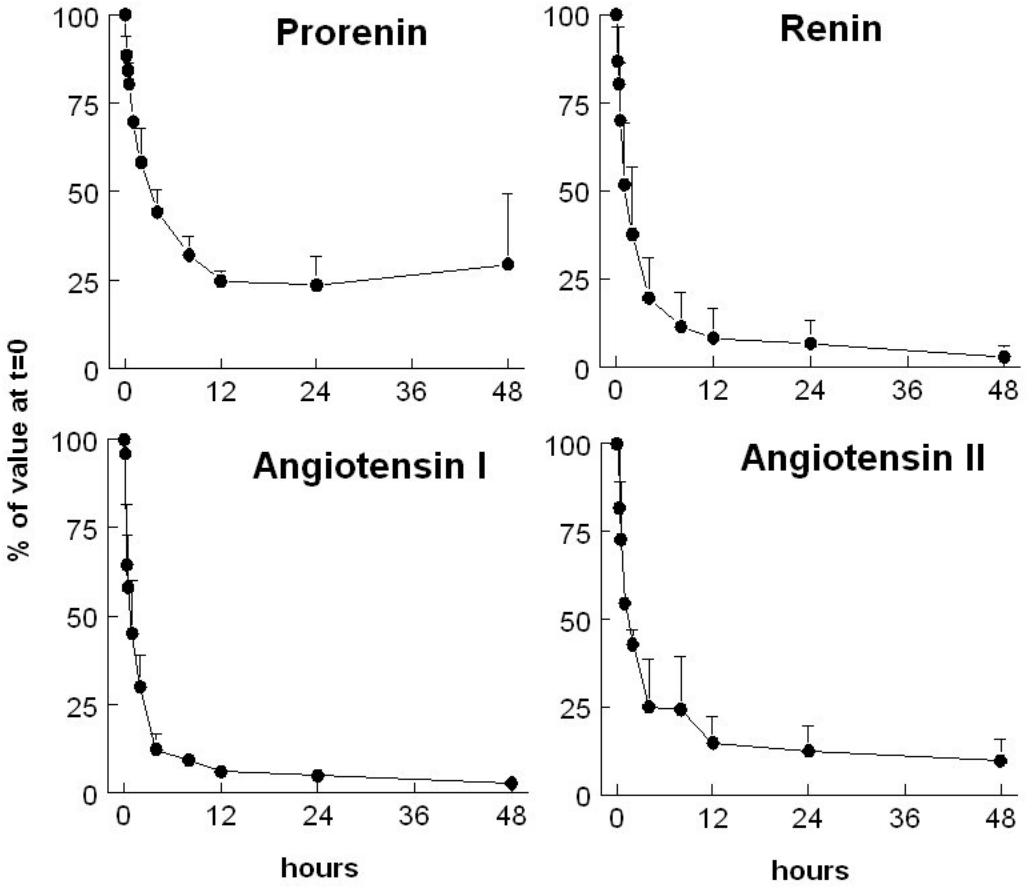

Figure 1. Plasma disappearance curves of prorenin, renin, angiotensin I and angiotensin II in patients after nephrectomy. Data (mean+SD of 4 subjects) have been expressed as a percentage of the values at t=0 (the time of renal vein clamping).

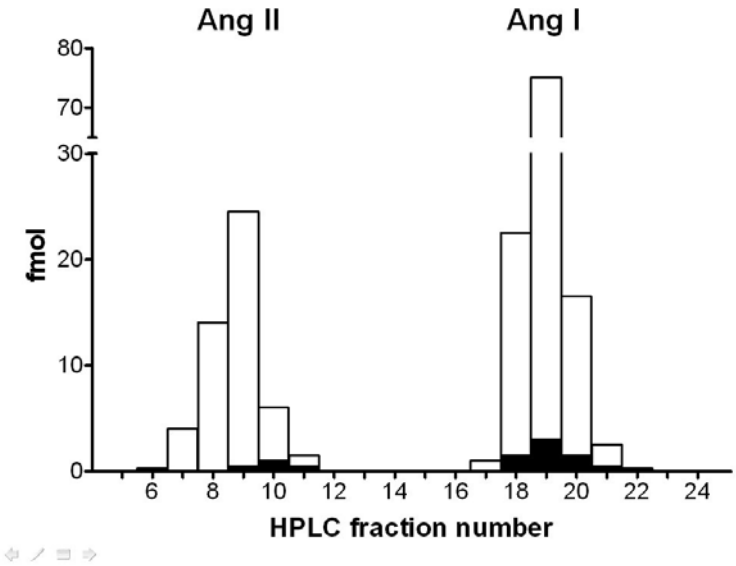

Figure 2. Angiotensin I and II levels found in HPLC fractions after SepPak extraction and HPLC separation of plasma obtained 30 minutes before (open bars) and 48 hours (closed bars) after nephrectomy.

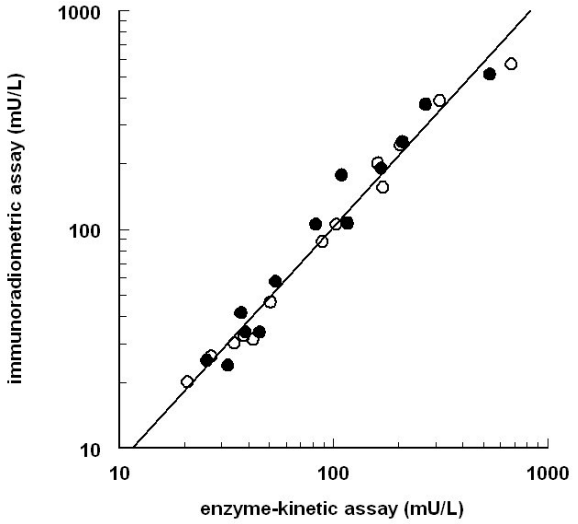

Figure 3. Comparison of the total renin concentration measured by enzyme-kinetic assay and by immunoradiometric assay in plasma samples obtained from 13 anephric subjects before (closed symbols) and after (open symbols) haemodialysis. The regression line is not significantly different from the line of identity (r=0.98; P<0.001).

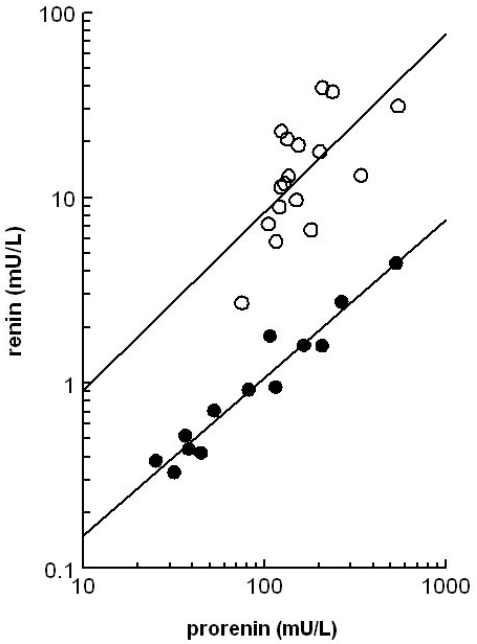

Figure 4. Relationship between renin and prorenin in healthy subjects (open symbols; log[renin]= 0.96xlog[prorenin]-1.00, r=0.64, P<0.01) and anephric subjects (closed symbols; log[renin]=0.59xlog[prorenin]-0.94, r=0.97, P<0.001).

prorenin are 0.15 and 0.075 L/min, respectively. The AUC's of kidney-derived renin and prorenin in this study were 17.7 and 234 U/L.min. With this information it is possible to estimate the total amount of kidney-derived renin and prorenin in the body (=AUC x clearance): 3 and 18 U, respectively. This is roughly ten times the total amount of renin and prorenin in blood. Apparently, therefore, 90% of both proteins is localized outside the circulation, i.e., is present at tissue sites. This could be an underestimation, in case a significant amount of renin and prorenin is also cleared outside the liver. However, when infusing ^{125}I-labeled prorenin in rats, the radiolabeled protein reached hepatic levels that were 10 times higher than those in the kidney, while <1% of the dose accumulated in other organs, and thus, the liver is by far the most important prorenin/renin-clearing organ in the body (31).

What might be the location of renin and prorenin outside the circulation? Both renin and angiotensinogen are known to enter the interstitium, reaching interstitial fluid levels in the rat heart that are comparable to those in blood (5,7,33). Interstitial fluid amounts to ≈15% of tissue wet weight, and thus an equal distribution of renin and prorenin over the extracellular fluid space cannot fully explain total body renin and prorenin. Indeed, the tissue levels of renin (expressed per g wet weight) are often as high as its levels per mL blood (2,3,27), which is impossible if tissue renin is limited to extracellular fluid and reaches concentrations in the interstitium that are identical to those in plasma. Therefore, an additional site must exist, which allows selective tissue accumulation of renin and prorenin. Nephrectomy studies in rats almost thirty years ago already suggested that arterial wall renin washout occurred more slowly than plasma renin washout, in full agreement with a 'renin binding site' in the vascular wall (34). Two likely candidates for such binding sites are the M6P receptor (9-11) and the recently discovered (pro)renin receptor (12). Studies with antagonists of these receptors would be required to definitely settle this issue. An argument in favor of the (pro)renin receptor is that prorenin binding to this receptor allows prorenin to display enzymatic activity (12,13), thus explaining why the majority of 'tissue renin' is usually active. However, an alternative explanation for the latter is prorenin-renin conversion during the tissue homogenization and extraction procedure.

Importantly, Ang I and Ang II disappearance from plasma following nephrectomy closely resembled renin disappearance, suggesting that kidney-derived renin (and not prorenin) is the major determinant of circulating angiotensins. Approximately 24-48 hours after nephrectomy, the renin, Ang I and Ang II levels in plasma were identical to those in subjects who had been anephric for years, that is they were ≤10% of the levels before nephrectomy or in healthy controls. This is in full agreement with previous studies in anephric humans (35). Prorenin did not decrease to such low levels. Its levels in anephric subjects were sometimes even in the normal range and remained stable over the years. Immunoreactive measurements confirmed that the prorenin levels measured by EKA truly resembled prorenin. Thus in humans, as has been suggested before (36,37), a major percentage of prorenin is of extrarenal origin. Although prorenin is enzymatically inactive, it is well-known that, depending on pH and temperature, prorenin is capable of undergoing a

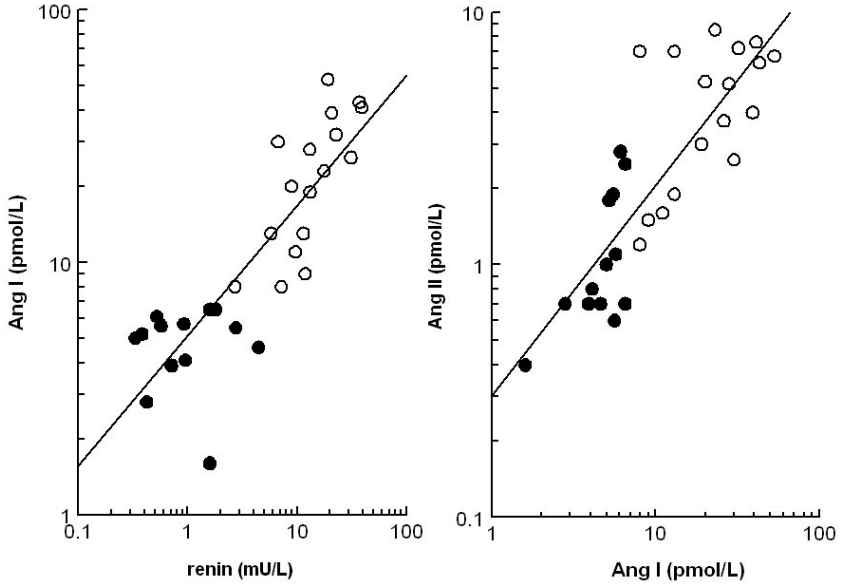

Figure 5. Relationship between renin and angiotensin I (left panel; log[angiotensin I]=0.62xlog[renin]+0.61, r=0.85, P<0.001) and between angiotensin I and angiotensin II (right panel; log[angiotensin II]=0.69xlog[angiotensin I]-0.35, r=0.75, P<0.001) in healthy subjects (open symbols) and anephric subjects (closed symbols).

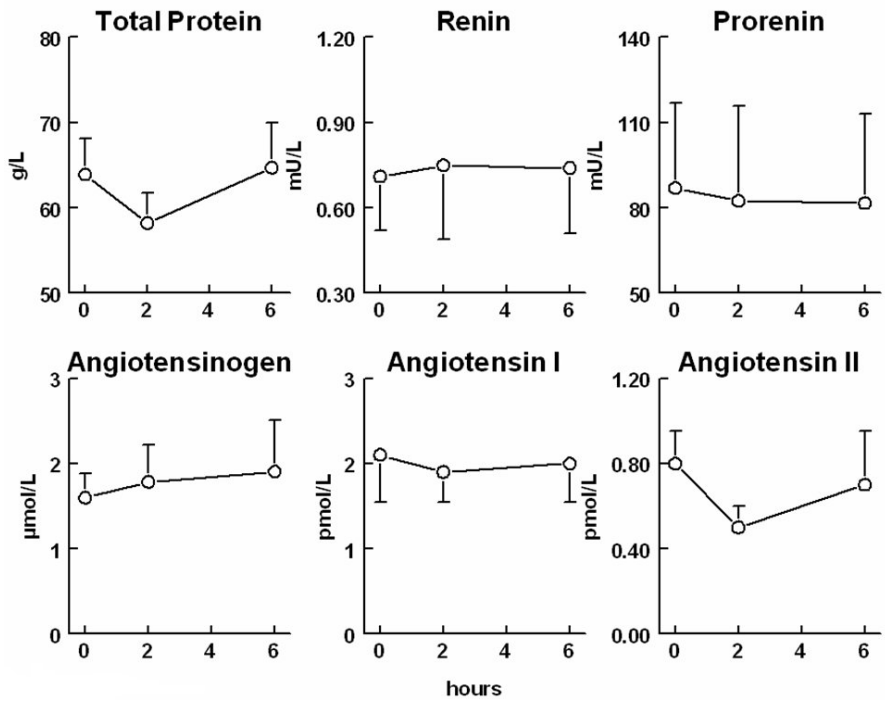

Figure 6. Plasma levels (mean+SD) of total protein, prorenin, renin, angiotensinogen, angiotensin I and angiotensin II in plasma of 5 anephric subjects given 50 mg captopril orally at t=0.

conformational change, involving the unfolding of the prosegment from the enzymatic cleft. Such unfolding is also known as 'non-proteolytic activation', and allows prorenin to become fully enzymatically active. Under *in vitro* conditions that mimick normal physiology as closely as possible (pH 7.4, 37°C), approximately 2% of prorenin is in this open conformation (10,13,38). The present study shows that 2% of total renin in anephric subjects is active (as opposed to >8% in healthy controls). Moreover, 'renin' in anephric subjects correlated highly significantly with prorenin, whereas normally the renin-prorenin relationship is much less strong (Figure 4) (18). Taken together, these

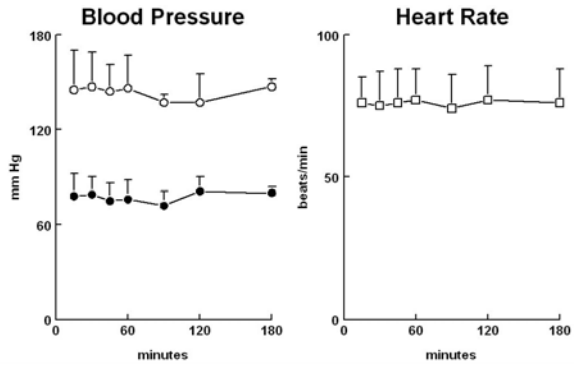

Figure 7. Systolic blood pressure (open circles), diastolic blood pressure (closed circles) and heart rate (squares) in 5 anephric subjects given 50 mg captopril orally at t=0. Data are mean+SD.

data suggest that 'renin' in anephric subjects is most likely open, non-proteolytically activated prorenin. Thus, the true renin levels in these subjects may actually be zero. This is in full agreement with human studies showing exclusive prorenin (and not renin) release from tissue sites (19,39) and animal studies showing no prorenin-renin conversion in the circulation following prorenin infusion or when overexpressing prorenin in the liver (25,30). A further possibility to attribute the Ang I-generating activity to prorenin is that (part of) plasma prorenin occurs in a truncated form, lacking a portion of the NH_2 terminus of the prosegment (40). Under such conditions, the prosegment will not fully cover the enzymatic cleft, and thus this truncated prorenin is enzymatically active.

The renin (=activated prorenin)-Ang I relationship in anephrics was identical to that in healthy controls, confirming that once prorenin has obtained its open conformation, its reaction with angiotensinogen is identical to that of renin. The Ang I-II relationship was also normal, suggesting that the ACE levels in anephrics are not altered. Captopril tended to reduce Ang II in anephrics, but the differences were, due to the closeness of the Ang II values to the detection limit, not significant. No changes occurred in either renin or Ang I, thereby confirming the idea that the renin rise following captopril is entirely kidney-dependent, and that the simultaneous Ang I rise is due to the rise in renin rather than its diminished conversion by ACE (41). The same applies to the RAS component changes that normally occur after haemodialysis (42). In contrast with a previous study (43), we did not observe a change in blood pressure following captopril exposure in anephrics. This may relate to the difference in experimental setup: in that study captopril was applied in the fluid-depleted state, one hour after haemodialysis, whereas in the present study captopril was applied immediately prior to haemodialysis, i.e., in the fluid-repleted state. A captopril-induced blood pressure drop in anephrics might be due to a suppression of prorenin-dependent Ang II generation and/or bradykinin potentiation. To exclude the latter, similar studies should be performed in anephrics using a renin inhibitor or an AT_1 receptor antagonist.

Studies with the (pro)renin receptor blocker HRP in animals have suggested that, under pathological conditions, prorenin contributes to tissue angiotensin generation, but (virtually) not to angiotensin generation in the circulation (14,23). Our data support the latter, since the majority of plasma angiotensins (>90%) disappeared in parallel with plasma renin following a nephrectomy, and plasma angiotensin levels remained low in anephrics, despite the continued presence of prorenin. No conclusions can be drawn on the contribution of prorenin to tissue angiotensin generation from our current results. In rats and pigs, tissue angiotensin levels dropped to or below the detection limit following a nephrectomy (3,44,45). As opposed to the situation in humans however, prorenin in these animals does not constitute 90% or more of total renin in plasma (3,23). Moreover, its levels dropped, at least in the pig, to almost the same degree as the renin levels after nephrectomy (3). Thus, one explanation for the lack of tissue angiotensin following a nephrectomy in animals might be a lack of both renin and prorenin. This would imply that in animals, more than in humans, prorenin is kidney-derived. An alternative, although less likely, explanation is that only kidney-derived prorenin contributes to tissue angiotensin generation, for instance because the glycosylation pattern of non-renal prorenin does not allow its penetration (via receptor-binding?) at tissue sites. Indeed, as has been discussed, glycosylation determines (pro)renin clearance (32), and M6P receptors do not bind non-glycosylated (pro)renin (10,11).

In summary, our study has revealed that kidney-derived renin and prorenin enter tissue sites in humans, and may thus contribute to local angiotensin production. A major percentage of prorenin is of extrarenal origin, and the strong correlation between 'renin' and prorenin in anephric subjects suggest that 'renin' in these subjects actually is non-proteolytically activated prorenin. Such activated, intact prorenin reacts normally with angiotensinogen, and thus, in combination with the doubled angiotensinogen levels and the unaltered ACE levels in anephric subjects, allows the generation of low levels of both Ang I and II in blood plasma following nephrectomy. To what degree prorenin also contributes to tissue angiotensin generation in humans remains to be determined.

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