Plasma kallikrein and plasmin as activators of prorenin: links between the renin–angiotensin system and other proteolytic systems in plasma

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Renin and prorenin
Renin is a circulating proteolytic enzyme acting at the physiological pH of blood; a circulating inhibitor of this enzyme has not been identified. Therefore, most studies on the biological significance of renin are based on the classical model of a circulating hormone. Renin, however, does not act on cellular receptors; it acts by virtue of its enzymatic activity.

Evidence has accumulated in recent years that a proportion of the renin in human plasma has little or no enzymatic activity. About 80% of the total potential renin activity of normal plasma is in this inactive form [1-4]. Possibly we are dealing here with a proenzyme comparable with the proenzymes that are activated by limited proteolysis in important physiological processes, such as coagulation, fibrinolysis, inflammatory responses and complement-mediated reactions. Usually, these processes are initiated in close contact with the blood vessel wall and not in circulating plasma. This could also apply to the renin–angiotensin system, so that circulating renin activity is not always a good index for the biological activity of the system. The traditional endocrinologist’s view on renin is perhaps too limited.

Inactive renin can be separated from active renin by ion-exchange chromatography [5, 6] and by affinity chromatography with pepstatin and certain dye ligands [7]. As will be discussed, inactive renin is converted (activated) in vitro into an active form of renin by various proteases. Plasma renin that is activated in vitro, naturally occurring active plasma renin and renin isolated from human kidneys as described by Haas, Goldblatt & Gipson [8] have similar properties, in terms of pH optimum, $K_m$ and inhibitory effect of pepstatin [9-12]. It is not certain, however, that inactive renin in plasma is a precursor of active renin in the course of its biosynthesis. Despite this uncertainty we will use here the term prorenin, because this form of renin has potential renin activity; it reacts, after activation, with natural renin-substrate to form angiotensin I.

Our knowledge of the biochemistry of prorenin is still preliminary. It may or may not represent a single molecular entity. A completely inactive prorenin has been isolated from plasma [7] but other forms of prorenin with some enzymatic activity before full activation may exist [13]. There is much debate on differences between prorenin, naturally occurring active renin and active renin generated in vitro, in terms of molecular weight and chromatographic behaviour [14–16]. Here we shall not discuss these questions but rather focus on the proteolytic enzymes, particularly the enzymes present in plasma, that are involved in the various pathways of prorenin activation in vitro. We shall consider the possible significance of these pathways in vivo.

Intrinsic, extrinsic and exogenous prorenin activators
The term activator is used here to designate proteolytic enzymes that are capable of activating prorenin in crude plasma or in partly purified fractions prepared from plasma. The use of this
term therefore does not imply that such an activator directly acts on prorenin.

**Intrinsic activators**

*Factor XII-kallikrein pathway.* The renin activity of human plasma is increased after 24 h-dialysis at pH 3.3 with subsequent restoration of pH 7.5. This also applies to human amniotic fluid. Lumbers [1] and Morris & Lumbers [17] were the first to show that this process of 'acid-activation', which is now known to be caused by conversion of prorenin into an active form of renin, is dependent on one or more proteolytic enzymes. The renin activity of plasma also increases during exposure of plasma to −4°C. Tatemichi & Osmond [18] have provided some evidence that this process of 'cryo-activation' depends on clotting factor XII (Hageman factor). Thus renin activity did not rise in Hageman-trait plasma. Experiments, in which serine proteinase inhibitors were added to plasma in the neutral phase after pH 3.3-dialysis, have subsequently shown that one or more serine proteinases are responsible for the increase in renin activity [19-21]. Studies carried out independently by Derkx, Bouma, Schalekamp & Schalekamp [22] and by Sealey, Atlas, Laragh, Silverberg & Kaplan [23] have demonstrated that both factor XII and prekallikrein (Fletcher factor) are involved here; the renin activity of Hageman-trait plasma and Fletcher-trait plasma did not rise in the neutral phase after pH 3.3-dialysis, whereas it rose after addition of trypsin, which seems to act on prorenin independently of factor XII and plasma kallikrein (Fig. 1).

![Activation of prorenin (mean values) at 4°C in normal plasma (n = 5), normal plasma from which plasminogen was removed by affinity chromatography on lysine-Sepharose as described by Deutsch & Mertz [29] (n = 4), factor XII-deficient plasma (Hageman-trait, n = 2) and prekallikrein-deficient plasma (Fletcher-trait, n = 2). Plasmas contained EDTA in a total concentration of 5 mmol/l. (a) Plasmas were dialysed against phosphate buffer of pH 7.5, made up 0.15 mol/l with NaCl, and stored at 4°C for various time periods as indicated. (b) Plasmas were treated with trypsin and with acid. Trypsin treatment: plasmas were dialysed against phosphate buffer of pH 7.5, made up 0.15 mol/l with NaCl, and incubated at 4°C with Sepharose-bound trypsin in a final concentration of 3000 a-N-benzoyl-L-arginine ethyl ester units/ml [22, 27] for various time periods as indicated. Acid treatment: plasmas were dialysed at 4°C for 24 h against glycine/HCl buffer of pH 3.3, made up 0.15 mol/l with NaCl, and incubated at 4°C with Sepharose-bound trypsin in a final concentration of 3000 a-N-benzoyl-L-arginine ethyl ester units/ml [22, 27] for various time periods as indicated. Acid treatment: plasmas were dialysed at 4°C for 24 h against glycine/HCl buffer of pH 3.3, made up 0.15 mol/l with NaCl, and incubated at 4°C with Sepharose-bound trypsin in a final concentration of 3000 a-N-benzoyl-L-arginine ethyl ester units/ml [22, 27] for various time periods as indicated. Acid treatment: plasmas were dialysed at 4°C for 24 h against glycine/HCl buffer of pH 3.3, made up 0.15 mol/l with NaCl, and incubated at 4°C with Sepharose-bound trypsin in a final concentration of 3000 a-N-benzoyl-L-arginine ethyl ester units/ml [22, 27] for various time periods as indicated. Acid treatment: plasmas were dialysed at 4°C for 24 h against glycine/HCl buffer of pH 3.3, made up 0.15 mol/l with NaCl, and incubated at 4°C with Sepharose-bound trypsin in a final concentration of 3000 a-N-benzoyl-L-arginine ethyl ester units/ml [22, 27] for various time periods as indicated.

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By adding active factor XII fragment (factor β-XIIa, mol. wt. 28 000) and highly purified plasma kallikrein (obtained by factor β-XIIa-activation of prekallikrein) to acid-pretreated Fletcher-trait plasma and acid-pretreated Hageman-trait plasma respectively, it could be demonstrated that kallikrein was the more direct activator of prorenin in this pathway. The factor XII-dependency of prorenin activation can be readily explained by the capability of this factor to convert plasma prekallikrein into kallikrein (Fig. 2).

C1-Esterase inhibitor and α2-macroglobulin are the most important inhibitors of kallikrein in plasma. The former is inactivated at pH 4.5-5.0. After treatment of plasma at this pH, however, little activation of prorenin is observed. α2-Macroglobulin is inactivated at pH 3.0-3.5 and this coincides with the generation of uninhibited kallikrein and the formation of active renin after pH has been restored [24]. It is possible that acid treatment is not only required for the generation of enough uninhibited kallikrein, and possibly other proteinases, but also for rendering the prorenin more susceptible to activation by these proteinases [25].

Plasmin. This also is an intrinsic activator of prorenin [26]. α2-Antiplasmin is the most important inhibitor of plasmin in human plasma. It is inactivated at pH 5.5-6.0 [24]. After dialysis of plasma at pH 4.0 for 24 h and subsequent restoration of pH α2-antiplasmin is not detectable any more, but there is little increase in renin activity. In contrast, large quantities of active renin are generated in pH 4.0-pretreated plasma after the addition of plasmin or the plasminogen activators, streptokinase and urokinase [27] (Fig. 1). By affinity chromatography on Cibacron-blue-Sepharose columns, using a phosphate buffer (0.02 mol/l) of pH 7.1 containing NaCl (0.2 mol/l) for elution, we have isolated from plasma a fraction which contained practically all the prorenin and plasminogen present in crude plasma. This fraction was virtually free of active renin and plasmin inhibitors. Addition of streptokinase, urokinase and highly purified plasminogen activator isolated from human uterine tissue [28] all led to the activation of prorenin. The plasminogen activators did indeed act via generation of plasmin; after removal of plasminogen by lysinesepharose affinity chromatography [29] the plasminogen activators had no effect on renin activity (Fig. 1).

Interactions of plasmin and the factor XII-kallikrein pathway. Experiments with purified factors have demonstrated that plasmin is capable of activating factor XII and that kallikrein can activate plasminogen. The factor XII-kallikrein pathway for prorenin activation, however, can proceed in the absence of plasmin [23]. Moreover, plasmin-mediated prorenin activation can proceed in the absence of factor XII and kallikrein [27] (Fig. 1). Kallikrein, via activation of prekallikrein by factor XII, and plasmin are the only intrinsic prorenin activators identified as yet, but there is no proof that these activators act directly on prorenin. Possibly, intermediate steps will be discovered.

Fig. 2 gives a schematic representation of the intrinsic pathways of prorenin activation and their possible connections with other proteolytic processes. The discovery that prorenin is activated via the factor XII-kallikrein pathway links the renin-angiotensin system with the

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**Fig. 2.** Factor XII-kallikrein and plasmin pathways of prorenin activation; connections with coagulation, fibrinolysis and kinin formation.
surface-mediated reactions of coagulation, fibrinolysis and kinin formation [30]. Both prekallikrein and factor XI circulate in plasma as complexes with the kallikrein-substrate, high-molecular-weight kininogen. Factor XII and kallikrein reciprocally activate one another in a positive feedback mechanism that also requires high-molecular-weight kininogen. Initial activation of factor XII may occur on exposure to negatively charged surfaces such as vascular basement membrane. Activated factor XII then triggers the cascades of coagulation and fibrinolysis. The biochemistry of these surface-mediated reactions has been reviewed [31].

Extrinsic activators

Glandular kallikrein. Two types of kallikrein are to be distinguished, plasma kallikrein and glandular kallikrein. High-molecular-weight kininogen is the preferred substrate for plasma kallikrein, and the product is bradykinin. Glandular kallikrein is found in exocrine glands and in the kidney. It is about equally active on low- and high-molecular-weight kininogen of plasma and the product is lysylbradykinin.

Sealey, Atlas, Laragh, Oza & Ryan [32] have demonstrated that glandular kallikrein, isolated from urine, is capable of activating prorenin at neutral pH in pH 3.3-pretreated human plasma. It has subsequently been shown that activation of prorenin by glandular kallikrein does not require prior acidification of prorenin [33].

Renal kallikrein has been localized to the distal tubule, possibly including the macula densa. This would favour an interaction with renin. Furthermore some kallikrein is released in the perfusate of isolated kidneys and small quantities of immunoreactive glandular kallikrein have been detected in plasma. However, immunochromological staining of kallikrein in the kidney was most intense on the luminal surface of tubular cells, whereas renin is localized in the afferent arteriole. This localization would indicate that these enzymes are released into urine and blood respectively, so that both proteins should never meet. For a more detailed discussion on renal kallikrein the reader is referred to Carretero & Scicli [34] and Keiser [35].

Vascular plasminogen activator. We have already mentioned that tissue plasminogen activator, which is probably similar or identical with the vascular activator (see the review by Collen [36]), is capable of initiating plasmin-mediated prorenin activation. This is an interesting observation since the release of vascular activator is increased by stimuli that are also known to increase the renin activity of plasma. We will come back to this in a later section of this review.

Exogenous activators

Trypsin is now often used for the conversion of prorenin into active renin in crude plasma in vitro as a first step in measuring prorenin [37–39]. The acid proteinases, pepsin and cathepsin D, also are capable of activating prorenin [40]. The effect of these exogenous proteinases on the renin activity of plasma does not seem to be mediated by the intrinsic prorenin activators, kallikrein and plasmin. We have already mentioned streptokinase and urokinase as exogenous initiators of plasmin-mediated prorenin activation.

Measurement of plasma prorenin

A direct assay specific for prorenin is not available. As yet, prorenin can only be measured after it has been converted into active renin and the conversion has to be complete to obtain a valid estimate. Kinetic studies on the activation of prorenin in crude plasma by trypsin, plasma kallikrein (after pH 3–3–dialysis, which causes inactivation of C1-esterase inhibitor and α2-macroglobulin) and plasmin (after pH 4–0–dialysis, which causes inactivation of α2-antiplasmin) have demonstrated that under optimal conditions the same plateau of renin activity is reached (Fig. 1). These data indicate that all the prorenin that can be converted by serine proteinase is indeed converted under these circumstances.

It is generally held that equimolar amounts of naturally occurring active renin and prorenin that is activated in vitro are equally active, but this has still to be proven. With this caveat, the difference between the concentrations of 'total' renin and naturally occurring active renin is considered an estimate of the prorenin concentration. A difficulty arises when the difference is either small or very large. For instance, the plasma concentration of renin in a normal individual in the recumbent position may be 20 μ-units/ml (with human kidney renin as a standard for expressing activity as described by Bangham, Robertson, Robertson, Robinson & Tree [41]) before activation in vitro, and 120 μ-units/ml after activation. The prorenin concentration is then calculated to be 100 μ-units/ml. After this individual has assumed the upright posture, his naturally occurring renin may rise by 10 μ-units/ml, a 50% change. If we assume that this increase is caused by prorenin into active renin conversion, and if we also
assume that equimolar quantities of naturally occurring active renin and prorenin activated in vitro are equally active, then the calculated concentration of prorenin will have decreased from 100 to 90 μ-units/ml, a 10% change, which is hard to detect.

**Activation of prorenin in vivo: does it occur?**

Parallel measurements of ‘total’ renin and naturally occurring active renin in plasma suggested a fall in prorenin in hypertensive subjects after head-up tilting and after intravenous injection of the potent vasodilator, diazoxide [3]; these stimuli caused a rapid rise of active renin in plasma. Similar responses to upright posture and intravenous injection of the potent diuretic, frusemide, have been observed by Weinberger, Aoi & Grim [42] and Munday, Noble & Richards [43] respectively. However, other investigators using the competitive angiotensin II antagonist, saralasin, or the angiotensin I-converting enzyme inhibitor, captopril, did not find that an increase of active renin was associated with decreased prorenin [4, 44].

Measurements of renal artery and vein samples before and after intravenous diazoxide in patients with renal artery stenosis have shown that the renal vein/artery ratio for measured prorenin on the side of stenosis fell from a value not significantly different from 1-0 to a value <1-0, while the renal vein/artery ratio for active renin was significantly >1-0 and rose after diazoxide [45]. A renal vein/artery ratio for prorenin <1-0 combined with an elevated renal vein/artery ratio for active renin on the affected side has also been observed in another series of patients with renal artery stenosis [46]. These findings are consistent with conversion of plasma prorenin into active renin as it is passing through the kidney. However, parallel increments of both forms of renin have been measured in the renal vein after isoprenaline and frusemide in anaesthetized pigs [47].

Administration of the β-adrenoceptor antagonists, propranolol and metoprolol, causes a decrease of active renin in plasma and there are reports that this is associated with increased prorenin [3, 48, 49]. Propranolol is also capable of preventing the rise of active renin and the fall of prorenin after diazoxide [45]. These findings suggest that the activation of prorenin might be under adrenergic control.

From the above studies it has also become clear that the plasma levels of naturally occurring active renin and prorenin are positively correlated under steady-state conditions both in normal subjects and in patients with different disorders. However, prorenin varies widely for a given level of active renin, possibly indicating varying degrees of activation.

Taken together there is suggestive evidence, but no proof, that activation of prorenin in vivo, inside or outside the kidney, really occurs.

**Possible role in vivo for plasma kallikrein and plasmin in prorenin activation**

It is often suggested that the coagulation and fibrinolytic cascades are continuously active in vivo, and that both systems are in a dynamic equilibrium to maintain an intact and patent vascular bed. A similar balance may exist between the renin-angiotensin system and the kallikrein–kinin system with their opposite effects on vascular smooth muscle tone. The presence of circulating angiotensins I and II and circulating bradykinin is good evidence that the renin and kallikrein systems are active in vivo. Moreover, a close and positive correlation between plasma renin activity and bradykinin has been observed in normal subjects with widely different intakes of sodium and potassium and with adrenocorticotropin hormone-induced hyperaldosteronism [50]. Conversely, the evidence for continuous low-grade coagulation and fibrinolysis is controversial.

It is possible that small quantities of kallikrein and plasmin are continuously formed not so much in circulating plasma, but rather on the inner surface of blood vessels. At this site the activators might be less accessible and also less susceptible to circulating inhibitors. Binding of factor XII and the high-molecular-weight kininogen–prekallikrein complex to a negatively charged surface is known to enhance the reciprocal activation of factor XII and prekallikrein, so that the activation process can proceed in the presence of inhibitors in the surrounding fluid phase. In an analogous way plasmin molecules that are bound to fibrin and are actively degrading fibrin are inactivated by α2-antiplasmin at a much slower rate than free plasmin, since they have both their active site and their α2-antiplasmin-binding sites protected from the inhibitor. The vascular plasminogen activator is also strongly bound to fibrin and this favours efficient activation of fibrin-bound plasminogen (see the reviews by Griffin & Cochrane [31] and by Collen [36]).

In the light of this knowledge we propose the following hypothesis on prorenin activation. Plasma prorenin is bound to the wall of certain blood vessels, perhaps including the glomerular
capillaries. When conditions are favourable for low-grade activation of prekallikrein or plasminogen, enough activator is generated to convert blood vessel-bound prorenin into active renin. There is indeed both direct and indirect evidence for the existence of vessel wall renin, which is capable of increasing smooth muscle tone via local angiotensin II formation (see the review by Swales [51]).

Evidence that plasma kallikrein might be important for the activation of prorenin in vivo is provided by measurements of naturally occurring active renin and trypsin-activatable prorenin in subjects with Fletcher-trait (prekallikrein-deficiency). Such measurements have shown that the proportion of plasma renin that is in the active form is abnormally low in these subjects, whereas prorenin is abnormally high [22, 23, 52]. Moreover, a positive correlation has been reported between the concentration of plasma prekallikrein and the proportion of plasma renin that is in the active form in normal subjects with varying degrees of sodium depletion [53].

Strenuous exercise, β-adrenoceptor stimulation and oestrogens are known to stimulate the release of vascular plasminogen activator. The increase in fibrinolytic activity observed after intravenous injections of the diuretic, frusemide, probably also depends on the release of vascular activator [54]. The same stimuli are known to increase the renin activity of plasma. Interestingly, the effect of frusemide on the fibrinolytic activity of plasma was not seen in nephrectomized subjects, and it has therefore been suggested that the diuretic induces the liberation of fibrinolytic activator from the vessel wall in the kidney.

It is conceivable that parallel stimulation of renin activity and fibrinolytic activity is somehow related to plasmin-mediated prorenin activation. Hedlin, Loh & Osmond [55] have found that the exercise-induced rise in plasma renin activity of women on oral contraceptive medication was associated with increased plasminogen activator and a rise in the proportion of active renin to prorenin. This proportion also increased after contraceptive medication alone. These data indeed indicate some link between fibrinolysis and prorenin activation.

Admittedly, the evidence so far that kallikrein and plasmin are involved in prorenin activation in vitro is only circumstantial, but the data that have been collected are encouraging and will stimulate investigators to pursue this new approach to the renin–angiotensin system.

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References
Kallikrein, plasmin and prorenin

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