Extrarenal Prorenin in Plasma Requires an Activator of Renal Origin

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SUMMARY Plasma prorenin may be of renal or extrarenal origin, and its conversion to renin may be catalyzed by renal or extrarenal enzymes. We tested the effect of bilateral nephrectomy and sham bilateral nephrectomy on plasma renin and prorenin in dogs, using low (3 mg/ml) and high (5 mg/ml) concentrations of trypsin to activate the prorenin. In the nephrectomized dogs, active plasma renin quickly disappeared, whereas plasma prorenin (inactive renin) increased by up to 300% during the first 24 hours after surgery, suggesting that it was released rapidly from a major extrarenal source but not converted to renin in the absence of the kidneys. In the sham surgery (control) group, plasma renin activity increased by up to 400% in the first 24 hours but returned almost to baseline by 48 hours, whereas prorenin remained at the preoperative value or fell below it. The quantity of prorenin varied greatly between the groups according to the time after surgery and the different concentrations of trypsin used. In the nephrectomy group, low and high trypsin levels resulted in similar prorenin values during the first 3 hours, but later on, the high trypsin level resulted in about twice as much prorenin. In the control group, high trypsin levels generally produced lower prorenin values than did low trypsin levels. Since trypsin is believed to interact with endogenous convertase enzymes in converting prorenin, a high requirement for it after bilateral nephrectomy suggests that removal of the kidneys causes a deficiency of such convertases. Conversely, the low requirement for trypsin after the stress of sham surgery suggests enhanced plasma convertase activity in the presence of the kidneys. Our observations point to the possible involvement of a special renal enzyme in the cascade of enzymes believed to participate in plasma prorenin activation. (Hypertension 8 [Suppl II]: II-84—II-88, 1986)

KEY WORDS • nephrectomy • convertase • trypsic activation • dog

THERE is growing evidence favoring the existence of prorenin, an inactive form of renin abundant in the circulation. Prorenin may be physiologically important if enough active renin and angiotensin are formed from it in regulated quantities to influence the fluid and electrolyte balance and systemic blood pressure.

It has generally been assumed that human plasma prorenin is normally derived in large part, if not entirely, from the kidneys, but there is also evidence of an extrarenal source, the precise nature and regulation of which remain to be established. Little work has been done on renal or extrarenal prorenin in animals because of delays in adapting the methods for its determination from human to animal plasma. Initial attempts to demonstrate prorenin in normal dog plasma yielded fairly low estimates or apparently negative results, but with methodological changes, we subsequently found higher proportions of prorenin-renin in dogs than in humans. It now appears that dogs and rats can be used for studies of prorenin-renin.

Bilaterally nephrectomized dogs develop extrarenal prorenin in their plasma very quickly. The plasma prorenin value rises above the preoperative level within 1 to 2 hours, continues to rise for up to 24 hours, and remains above the preoperative level for at least 120 hours. This contrasts with the rapid disappearance of renin itself, indicating very little, if any, release of renin from an extrarenal source, or formation of it from extrarenal prorenin in the plasma.

Such a remarkable development of plasma prorenin immediately after nephrectomy has also been reported in rats, and we have been able to confirm it (P. Ioannou and D. H. Osmond, unpublished data, 1985). Thus, there is mounting evidence from several laboratories in favor of extrarenal prorenin production in

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humans and animals by a source that can rapidly supply the plasma with prorenin, but not renin.

Human plasma after nephrectomy reportedly has subnormal prorenin convertase activity, enough to limit the activation of its prorenin content. The evidence suggests that a given prorenin measurement reflects at least three interacting factors: the actual quantity of prorenin available, the potency of the endogenous convertase mechanism, and the efficacy of the applied activation procedure (acid, cold, or trypsin), believed to act in cooperation with the endogenous convertase. A strong exogenous stimulus (e.g., a high trypsin concentration) can apparently compensate for a weak endogenous mechanism, and vice versa.

The purpose of this study was threefold. First, we sought additional evidence regarding the cooperative relationship between exogenous and endogenous convertase mechanisms of prorenin activation. Second, we wished to define in greater detail the time course of plasma prorenin development in dogs after bilateral nephrectomy. Third, in view of earlier questions, we sought more evidence that postnephrectomy prorenin has an extrarenal rather than residual renal origin, and is not merely a consequence of the stress of surgery.

Materials and Methods

Eleven normal mongrel dogs (8 male and 3 female) weighing 9 to 18 kg were maintained on a standard laboratory diet.

Eight of the dogs were bilaterally nephrectomized through a single midline incision, under halothane anesthesia. The remaining three underwent a "sham" nephrectomy in which the kidneys were exposed and handled but not damaged or removed.

Blood samples were collected by venipuncture from quiet conscious dogs before surgery and at 1, 3, 6, 24, and 48 hours thereafter. The anticoagulant was 15% ethylenediaminetetraacetate, ammonium form, 30 μl per milliliter of blood. Samples were placed immediately on ice and centrifuged at 2000 g, 4°C, for 20 minutes. The plasma was separated and stored at −60°C until further assay.

Plasma renin activity was determined as the rate of angiotensin I generation during incubation at 37°C, pH 6.0, for 15 and 30 minutes, with added angiotensinase inhibitors. The angiotensin was determined by radioimmunoassay (125I-labeled angiotensin I kit, Du Pont–New England Nuclear Medical Diagnostics Division, North Billerica, MA, USA), and the plasma renin activity expressed as nanograms of angiotensin I per milliliter of plasma per hour.

Plasma prorenin was converted to renin by trypsin activation. Trypsin (T-8253, type HI, 10,000 BAEE units per milligram of protein; Sigma Chemical, St. Louis, MO, USA) was dissolved in 0.002N HCl and was added to plasma to produce a final concentration of 3 or 5 mg per milliliter of plasma. These trypsin concentrations, here called "low" and "high," respectively, induce moderate and nearly maximal plasma prorenin activation in normal dogs.

Plasma samples were incubated with trypsin at 23°C for 10 minutes, and the reaction was terminated by adding an excess of lima bean trypsin inhibitor (T-9378, Sigma), as described previously. Plasma renin activity was then assayed, and prorenin estimated as the difference between the values obtained before and after trypsinic activation.

Statistical comparisons were carried out with paired or unpaired t tests and a two-tailed distribution of t values. A least-squares means (postmodel) analysis was also carried out on some of the data, using the General Linear Models Statistical Analysis System (GLM-SAS; Statistical Analysis System Institute, Cary, NC, USA).

Results

In the control dogs, which underwent sham bilateral nephrectomy, plasma renin activity (active renin) more than doubled within 3 hours after surgery and practically doubled again by 24 hours, slowly dropping toward the preoperative value by 48 hours (Figure 1).

In the nephrectomized group plasma renin activity essentially disappeared within 3 hours after surgery and did not reappear throughout the 48 hours of the experiment (see Figure 1).

With a trypsin concentration of 3 mg per milliliter of plasma (low trypsin), prorenin was estimated at 16 ng/ml/hr before surgery. Within 1 hour, prorenin rose only to 22 ng/ml/hr in the control dogs, with little change thereafter (Figure 2). In the nephrectomized dogs, the preoperative prorenin value happened to be higher (26 ng/ml/hr) but increased dramatically to 68 ng/ml/hr within 1 hour, stayed near that level for 24 hours, and then declined slowly during the final 24 hours.

Significant differences from these patterns were observed when the higher trypsin concentration (5 mg per milliliter of plasma) was used. In the sham surgery group, less prorenin was detected during most of the postsurgical period, especially at 24 hours, with a re-

![Figure 1. Plasma renin activity in dogs with time after sham or true bilateral nephrectomy (2NX). Values are expressed in terms of angiotensin I, ng/ml/hr (means ± SEM).](#)
turn to the original value by 48 hours (see Figure 2). In the nephrectomized group high trypsin generally had the opposite effect. Initially, at 1 and 3 hours, it resulted in essentially the same prorenin values as low trypsin, but later on, it produced much higher prorenin values ($p < 0.025$ at 24 hours, $p < 0.001$ at 48 hours).

The nature and extent of these differences are highlighted by the histogram in Figure 3. High trypsin resulted in 20 to 60% less prorenin than the initial value in the sham surgery group ($p < 0.005$ at 1 and 6 hours). After bilateral nephrectomy, high trypsin resulted in prorenin estimates that were up to 167% higher than before surgery ($p < 0.025$ at 24 hours), reflecting some plasma change that greatly influenced its response to activation.

Discussion

In dog plasma prorenin persists and increases quickly with time after bilateral nephrectomy (see Figure 2), confirming our earlier observations. The nature and source of this prorenin are unknown, but it behaves like normal prorenin, in that it is activated by the same trypsin procedure as is used in normal dog plasma. The renin produced from it reacts with endogenous plasma angiotensinogen to produce angiotensin I that is detectable by our highly specific radioimmunoassay technique. Thus, in terms of assay but not necessarily chemical structure, plasma prorenin after bilateral nephrectomy seems to be indistinguishable from normal prorenin.

The observed rise in plasma prorenin after bilateral nephrectomy may have been due to residual renal or extrarenal prorenin, or to the rapid accumulation of new extrarenal prorenin. The present evidence favors the latter explanation, because our control dogs showed only a trivial rise in prorenin after sham surgery (see Figure 2), whereas the nephrectomized dogs exhibited a threefold rise in prorenin during the first 6 hours after surgery. Such a dramatic increase seems difficult to attribute only to accumulation and diminished clearance of residual renal prorenin.

Very little, if any, of the prorenin present after bilateral nephrectomy was converted to renin in vivo. The plasma renin activity dropped almost to zero within 3 hours and remained at that low level throughout the 48-hour period (see Figure 1). This contrasts with the almost fourfold rise observed in the control dogs at 24 hours (see Figure 1), as a result of stimulated renal release or systemic conversion of prorenin.

Our observations indicate that the presence of the kidneys is associated with a high-renin, high-convertase state, and their absence with a low-renin, low-convertase state, as previously suggested by studies in nephrectomized humans and animals. The argument hinges on the evidence that trypsinic and other forms of activation in vitro appear to be influenced by the action of endogenous convertase enzymes, such that when their activity is high, less trypsin is required to achieve a given level of prorenin activation. Conversely, when endogenous convertase activity is low, more trypsin is required to produce that amount of activation.

Thus, in dogs that have undergone sham bilateral nephrectomy (high renin and convertase), the prorenin estimated by high trypsin is less than that estimated by low trypsin, at almost all intervals after surgery (see Figure 2). This suggests that high trypsin is in excess relative to the convertase available and, therefore, that optimal activating conditions have been exceeded. Prorenin, alone or with other reactants such as the...
renin derived from it, is probably destroyed by excess trypsin, causing the prorenin estimate to drop.\textsuperscript{12}

In nephrectomized dogs (low renin and convertase), the prorenin estimate with high and low trypsin is the same at 1 and 3 hours after surgery (see Figure 2). Thereafter, the prorenin estimated with high trypsin becomes much higher — essentially twice as high at 24 and 48 hours (see Figure 2). This suggests that as endogenous convertase drops with time after bilateral nephrectomy, the high trypsin is better tolerated and becomes more effective in maximizing the prorenin estimate. Concurrently, the low trypsin becomes progressively less effective in estimating the available prorenin.

This relationship is even more clearly discerned in data representing the percentage of change in prorenin values with time after surgery (see Figure 3), the baseline being the prorenin estimated with high trypsin before surgery. In plasma from the control dogs, 60\% less prorenin was estimated to be present 24 hours after surgery, whereas in plasma from the nephrectomized dogs, nearly 170\% more prorenin was estimated to be present at 24 hours with the same high trypsin concentration (see Figure 3).

These observations can be further clarified by referring to typical trypsin titration curves,\textsuperscript{11, 12, 20} based on trypsin with essentially the same specific activity as in the present study (see Methods). Figure 4 shows that in human plasma the maximum prorenin estimate is generally obtained using trypsin at a concentration between 1.0 and 1.5 mg per milliliter of plasma, whereas in dog and rat plasma the optimum level is much higher (i.e., 4–5 mg/ml).

It is important to note that submaximal prorenin values are obtainable on either side of the peak estimate, for very different reasons. On the left, the prorenin is underestimated because of inadequate activation, whereas on the right, it is underestimated because of destruction.\textsuperscript{12, 13} For dog plasma, trypsin at 3 mg/ml is suboptimal, and a concentration of 5 mg/ml is somewhat excessive, such that in plasma with a high endogenous convertase content there could be destruction of prorenin and other reactants, thereby resulting in lower estimates.

These concepts are further elaborated in Figure 5, with three different hypothetical plasma samples that have the same prorenin content but different amounts of convertase. In these plasma samples the same prorenin concentration can be estimated with three different concentrations of trypsin. In the plasma with the highest endogenous convertase content (left curve, representing sham bilateral nephrectomy), a low trypsin level is fully effective, whereas in the plasma with the lowest convertase content (right curve, representing bilateral nephrectomy), a much higher trypsin concentration is required to achieve the peak. Where the left and right curves intersect with the middle curve (representing normal plasma), the same estimate of prorenin is obtainable on either side of the peak, with slightly low or high concentrations of trypsin, respectively. This model conforms to the present and previous data.\textsuperscript{12, 13}

Our hypothesis of a special renal convertase appears to extend earlier evidence of convertase enzymes, of
unknown identity and origin, that are inhibited by serine and other protease inhibitors. Similarly, the identification of factor XII, kallikrein, and plasmin in prorenin activation suggests that more than one enzyme is involved and that the proposed renal conversion may be neither a single enzyme nor the only activator. Instead, renal conversion could be one or more key enzymes limiting the action of other, nonrenal enzymes in a cascade of reactions involved in the activation of prorenin.

In conclusion, a generous extrarenal source of prorenin appears to supply the plasma in dogs immediately after nephrectomy, quickly raising their prorenin level above the preoperative value. However, little or no active renin is formed from it, suggesting that conversion of prorenin to renin is impaired in vivo because of the disappearance of one or more important convertases of renin origin. In dogs that have undergone sham nephrectomy there is a marked rise in active renin, with essentially no rise in prorenin, suggesting possible stimulation of the convertase mechanism and enhanced conversion of prorenin to renin. Such stimulation is also suggested by the fact that their prorenin is more effectively converted to renin in vitro by a low concentration of added trypsin, whereas in plasma from nephrectomized dogs high trypsin is much more effective. Thus, after sham surgery dog plasma contains high levels of renin and convertase, whereas after actual nephrectomy, the levels are low. Our data further suggest that prorenin estimates reflect the available quantities of both prorenin and endogenous convertase, as well as the "goodness of fit" of added trypsin. In this sense they are analogous to plasma renin activity, which also represents not just renin itself but rather the expressed renin activity, reflecting all the influences that affect the generation of angiotensin.

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