Does Prorenin Exert Angiotensin-Independent Effects In Vivo?

A.H. Jan Danser

Thirteen years ago, Véniant et al. made the intriguing observation that targeted expression of rat prorenin to the liver under the control of the human α₁-antitrypsin promoter increased plasma prorenin levels 600-fold in male transgenic rats and caused cardiac hypertrophy, myocardial fibrosis, and severe renal lesions by 20 weeks of age. Renal renin content in these rats decreased, and plasma angiotensin (Ang) I–generating activity increased, suggesting that (local) prorenin activation had resulted in Ang II generation and, thus, a negative feedback effect on renal renin release. Yet, blood pressure was unaltered. The authors, therefore, concluded that long-term exposure to prorenin is vasculotoxic. Whether this toxic effect involved Ang II remained unexplored.

The discovery of the “(pro)renin receptor” subsequently provided the putative missing link: apparently, prorenin (like renin) was able to bind to a receptor and to even exert direct, Ang-independent effects via this receptor. Such effects included the production of transforming growth factor-β, and plasminogen-activator inhibitor 1 and, thus, possibly fibrosis. Moreover, blockade of this receptor with an antagonist called handle region peptide prevented these prorenin-induced effects in vivo, even in Ang II type 1 receptor knockout animals. Clearly, therefore, the vasculotoxic effects of prorenin now had a firm basis and did not appear to involve Ang.

Nevertheless, not all studies supported this view. The effect of handle region peptide on prorenin binding and signaling was questioned both in vitro and in vivo. Virtually all of the studies investigating prorenin’s (pro)fibrotic effects in vitro applied nanomolar concentrations of the enzyme, despite its picomolar concentrations in vivo. Thus, the physiological relevance of such findings is questionable. In addition, 2 recent studies in transgenic rodents display, respectively, 180- and 13- to 28-fold elevated levels of prorenin observed an increase in blood pressure, but no glomerulosclerosis or cardiac fibrosis, in full contrast to the results by Véniant et al. In such rodents, angiotensin-converting enzyme inhibition rapidly normalized blood pressure, whereas handle region peptide was without effect. Moreover, animals expressing active site-mutated prorenin (not capable of generating Ang I) showed no change in blood pressure. Thus, on the basis of these studies it appeared that prorenin acted through the generation of Ang to raise blood pressure. In support of enhanced Ang generation, the prorenin-expressing animals had low renin levels, suggestive of a negative feedback effect of Ang II on renal renin synthesis. The latter observation had been made by Véniant et al. as well, although the rise in Ang II in that study, if present, had not resulted in a rise in blood pressure.

In the present issue of Hypertension, Campbell et al. have re-evaluated the transgenic rats of the 1996 article, focusing in particular on the mechanism of the phenotype. The parental line for these animals was the original 85-26 line described in the article by Véniant et al. Yet, in contrast to the initial report, the authors now find that the male transgenic rats were hypertensive by 3 months of age, developed only modest renal lesions and cardiac fibrosis after 6 months of age, and had a normal aortic wall thickness. Cardiac hypertrophy was present from the age of 3 months on, presumably as a consequence of the rise in blood pressure. In other words: these animals resembled the transgenic prorenin-overexpressing animals reported recently by Peters et al. and Mercure et al. Plasma prorenin levels in the current transgenic rats were 1000-fold higher than in wild-type litters, whereas plasma and tissue (kidney) Ang II levels were no different from wild-type levels. Kidney renin levels were suppressed by 90%, in agreement with the negative feedback of prorenin-dependent Ang II generation on renal renin release. Indeed, the Ang I–generating activity of plasma was above normal, suggesting that somehow the exceptionally high prorenin levels were capable of keeping Ang I generation at a high level. Importantly, binephrectomy yielded the expected results in wild-type rats (a reduction in plasma renin activity by 80% and a reduction in plasma Ang II by 90% after 24 hours) but increased the plasma Ang I–generating activity and Ang II levels in transgenic rats. The latter probably relates to the acute phase response of the α₁-antitrypsin promoter of the prorenin transgene, evidenced by the 6-fold rise in prorenin after binephrectomy.

The authors are to be complimented for their courage to publish these data after the initial, opposite report. Moreover, the detailed biochemical measurements, in particular, those of Ang II, provide important additional information that was not yet clear from the studies by Peters et al. and Mercure et al. Now that we have all of the data, a more complete picture emerges on the role of prorenin.

Campbell et al. are unable to explain the difference in phenotype. Differences in genetic background, diet, and/or animal housing are briefly mentioned, as well as the use of
anesthesia in the first study to measure blood pressure. Nevertheless, a vasculotoxic, Ang-independent effect of prorenin now seems unlikely. Its effects on blood pressure, cardiac and vascular hypertrophy, fibrosis, and glomerulosclerosis are mediated via Ang II. This is also supported by our recent observation that the blood pressure of double-transgenic rats expressing both the human (pro)renin receptor and human renin is not different from that of transgenic rats expressing the human (pro)renin receptor only (Dr. Michael Bader, unpublished data, 2008). Whether all of the prorenin-induced effects that have been described thus far (including its effect on urinary acidification) are Ang dependent remains to be proven.

Although all 3 of the studies in prorenin transgenic rats imply that prorenin expression somehow resulted in Ang II generation, thus suppressing renal renin release, it remains unclear how this might have occurred. No evidence was obtained for significant prorenin-renin conversion in any of the 3 studies, in agreement with the fact that this does not occur at extrarenal tissue sites. This leaves the modest endogenous activity of prorenin and/or prorenin binding to its receptor (Figure). The latter binding not only allows prorenin to act as an agonist (and to induce the above-described Ang-independent effects), but it simultaneously induces a conformational change in the prorenin molecule so that it is capable of reacting with angiotensinogen.

Regarding the endogenous activity of prorenin, it is important to realize that prorenin occurs in a “closed” and an “open” conformation (Figure). Open prorenin is enzymatically fully active and can be recognized by monoclonal antibodies that are specific for the active site. This so-called “nonproteolytic activation” of prorenin (Figure) is attributed to unfolding of the prosegment from the enzymatic cleft. Such unfolding occurs particularly at low pH and low temperature. It probably also occurs after binding to the (pro)renin receptor. Under physiological conditions (pH 7.4; 37°C), however, <1% to 2% of human prorenin is in the open form.

Based on renin and prorenin measurements, Campbell et al conclude that, in their transgenic rats, 0.03% of prorenin is in the open form (ie, displays enzymatic activity). Under such circumstances, 50,000 prorenin molecules would generate as much Ang I as 15 (50,000*0.03/100) renin molecules, and, thus, as explained in the article, the 1000-fold rise in plasma prorenin in the transgenic rats is roughly sufficient to generate the same amount of Ang I as plasma renin in the wild-type rats. Yet, it has been known for years that plasma Ang I–generating activity alone is insufficient to generate all of the Ang I in plasma and that significant uptake of circulating (pro)renin is required to explain the even higher levels of tissue Angs. The renal Ang II levels in the prorenin transgenic rats were as high as those in wild-type rats, despite the 90% decrease in renin content. This suggests a role for circulating prorenin as a contributor to tissue Ang generation.

Importantly, in this regard, Prescott et al have generated mice expressing human prorenin in the liver and human angiotensinogen in the heart. These mice displayed increased cardiac (but not plasma) Ang I levels. Because the renin-angiotensinogen reaction is highly species specific (ie, mouse renin does not react with human angiotensinogen and vice versa), this approach elegantly demonstrates that, apparently, circulating prorenin is taken up in the heart and reacts locally.
with angiotensinogen. Because the same phenomenon occurred when expressing a noncleavable variant of prorenin, the activity of prorenin at the tissue level clearly did not depend on proteolytic removal of the prosegment. Whether it involves a receptor, for example, the (pro)renin receptor, to allow sufficient tissue Ang generation remains to be proven. Future studies should, therefore, carefully evaluate the uptake and activation of prorenin at tissue sites. Such studies are technically challenging because of the risk of inadvertent prorenin activation during tissue preparation. In addition, the capability of the handle region peptide to prevent glomerulosclerosis and cardiac fibrosis should be re-evaluated in light of the new findings.

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