How Should Human Plasma Prorenin Be Determined?

Dr. P.L. Drury has focused attention on incomplete cryoactivation (CA) as a source of confusion in the estimation of human plasma prorenin. In essence, Dr. Drury cites evidence that CA may proceed for as long as 3 months (or longer) and argues that shorter intervals determine an unspecified proportion of an unknown total. He argues in favor of determining total prorenin, but recognizes that, among other things, the time required is prohibitive.

First, we must question whether CA takes so long to be completed. It has been shown that at temperatures below 0°C, such as -5° or -4°C, CA plateaus after 2 weeks or so, provided renin activity is measured and not renin concentration (PRC, with addition of substrate). Even at +4°C, the temperature at which CA was originally demonstrated, near-maximal activation is attained within 30 to 60 days. We have reported that the addition of impure substrate may contribute prorenin “convertases” and prorenin itself, which amplify the estimations and may even give false positives. If so, the lack of a plateau after 3 months, observed using PRC methodology, is probably due to the addition of such contaminants. Using methodology for renin activity (PRA, no added substrate), we have not encountered this problem.

Second, even in cases where a CA plateau is reached, the apparent total prorenin varies considerably in different studies, allowing for two interpretations. The actual prorenin content may vary greatly among individual plasmas, and/or the efficacy of CA varies so that all the prorenin present is not exposed at the plateau. In other words, the plateau does not represent the true total concentration of prorenin but only that portion of it that happens to be exposed by CA.

Thus, we have recorded a rise of PRA from approximately 5 to 30 ng/ml/hr, representing a prorenin estimate of 25 for normal human plasma by CA at -4°C for 14-28 days. In comparison, Drury and Edwards reported a PRA rise from about 1 to 3 ng/ml/hr after 15 days of CA at -5°C. In contrast, Matsunaga et al. using PRC methodology (with the addition of sheep substrate) reported a rise from about 8 to 37 ng/ml/hr after CA for 7 days at -4°C — not far removed from our values for PRA but without reaching a plateau. Sealey et al. reported rapid CA within 4 days at -2°C, with the PRA rising from about 0.8 to 4 ng/ml/hr; in the subsequent 4 days, there was a further rise of only about 0.5 ng/ml/hr, suggesting that a plateau had been reached. Clearly, there is appreciable variability from laboratory to laboratory in the height of the prorenin plateau, the prorenin concentration it represents, and the time and conditions required to reach it.

Incidentally, a similar point may be made with respect to tryptic activation (TA). The concentration of trypsin added and the incubation protocol both determine the quantity of prorenin that is measured.

Third, we seriously question whether “total prorenin” is required for comparative studies. We have demonstrated that CA, at least, is a function of the plasma’s content of both prorenin and its activating enzymes or “convertases.” The estimate of the first is substantially influenced by the second. For instance, a deficiency of “convertase” in postnephrectomy human plasma results in prorenin values that are lower by CA than by suitable TA. Just as PRA is not a “complete” measurement but still gives valuable comparative data for clinical and investigative purposes, so “partial prorenin” determined under specified, consistent conditions may provide equally valuable data. Just as PRA reflects not only the “activation” of renin itself but also that of substrate and the combined influences of any inhibitors or activators of the reaction, so may “partial prorenin” adequately reflect the status of the prorenin-renin system.

Fourth, we present data that underscore the problem of equating the activation plateau with total prorenin. In a plasma pool obtained from 6 healthy men, CA at -4°C produced a near-maximal prorenin value of 13.6 ± 2.6 ng/ml/hr after only 6 days (fig. 1). This
was slightly higher than the 8.4 ± 1.0 ng/ml/hr determined with a near-optimal concentration of trypsin (1.0 mg/ml plasma, fig. 1). Active renin (basal PRA) was 6.3 ± 0.5 ng/ml/hr. When the same plasma pool was trypsinized with 1.0 mg trypsin/ml and then CA for 6 days at -4°C, the prorenin value rose to 29.9 ± 2.2 ng/ml/hr, which was 8 ng above the calculated sum of the effects of CA and trypsin taken separately. Obviously, pretrypsinization caused CA to increase by about 58%, and the previously observed estimates of "total prorenin" which could be obtained by either CA or TA alone can be amplified. It is not clear whether each method activates a separate pool of prorenin or whether TA merely facilitates the effect of cold on one and the same pool. We suspect the latter.

In conclusion, most of our knowledge of prorenin, based on over 400 publications, derives from experiments that determined partial rather than total prorenin in plasma and other tissues. We owe a great deal to such methodologies. But both partial and total prorenin, whether activated by acid, cold, or added enzymes, remain derivative measurements twice removed, i.e., prorenin → renin → angiotensin I. Only angiotensin I is directly determined in most laboratories. Moreover, each arrow represents reactions influenced by several variables that are not necessarily well controlled. It is important to refine present methods. It is even more important to develop new direct methods for prorenin and renin.

References

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(Hypertension 5: 805-806, 1983)
How should human plasma prorenin be determined?
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Hypertension. 1983;5:805-806
do: 10.1161/01.HYP.5.5.805

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