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# Studies on the Characterization of Pure Submaxillary Gland Renin[1] (38293)

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In a previous report, we described the isolation, in stable and pure form, of a family of renin-like enzymes from the submaxillary gland of the adult male mouse (1). The chemical and physical properties of two of the enzymes, renin A and C, were examined. They appeared to be the purest preparations with renin-like activity from any source yet reported. Molecular weights of 43,000 and 36,000–37,000 were obtained by gel filtration and by equilibrium sedimentation, respectively. Renin A was obtained in crystalline form. The amino acid compositions of the two enzymes were very similar and both reacted with synthetic renin substrate to generate angiotensin I (1).

In this report we describe some of the pharmacologic, physiologic, and kinetic characteristics of these pure renin-like enzymes.

*Materials and Methods. Preparation of submaxillary renins (SR).* The isolation and purification of both renin A and renin C from the submaxillary glands of male mice was performed as described previously (1). In brief, after extraction in water, ammonium sulfate fractionation, and five steps of column chromatography on Sephadex G-100, diethylamino ethyl cellulose and carboxymethyl cellulose, five pure isoenzyme fractions were separated. The major fraction (Renin A) contained approximately 80% of the total SR activity. The second major fraction (Renin C) contained 10% and the remaining three fractions (Renin B, D, and E) contained 1–2% of total renin activity. The purity of renins A and C was established by disc electrophoresis, ion exchange chromatography, ultracentrifugation and immunodiffusion (1). For the studies reported, dilutions of the renin were made with 0.9% bovine serum albumin.

*Effect of SR on blood pressure.* Rats were used as experimental animals. They were nephrectomized 24 hr prior to the experiments and were pretreated with pentolinium and phenoxybenzamine. Under pentobarbital anesthesia, a catheter was inserted into the carotid artery and connected to a transducer for pressure recording on a Beckman recorder. Two catheters were inserted into one jugular vein for injections of pure SR samples and standard solution of angiotensin II. Only animals responding to 0.4 ng of angiotensin II with a rise of at least 8 mm Hg in mean blood pressure were used. After the intravenous injection of 0.4 and 0.8 ng of standard angiotensin II, 0.6 ng of submaxillary renin was injected intravenously followed by infusion of the same doses of angiotensin II as before.

*Preparation of plasma renin substrate.* Plasma from nephrectomized rats and purified renin substrate obtained from the same plasma were used as substrates, in order to study the effects of pH, enzyme concentration, time of incubation, and substrate concentration on the rate of angiotensin formation by the pure submaxillary renin. The animals were bilaterally nephrectomized and 48 hr later the blood was drawn into cold siliconized tubes containing 0.3 ml of 9% EDTA, the plasma was separated by centrifugation in the cold and then it was frozen until use. This plasma was then used as renin substrate. Prior to its use it was tested for the presence of renin (2) and it was found to contain no detectable amounts of plasma renin activity (PRA). For the isolation of renin substrate a procedure previously reported (3, 4) was employed. Pure SR was incubated with nephrec-

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tomized plasma or purified renin substrate in pyrophosphate buffer and the generated angiotensin was measured as PRA by a method previously reported (2). It is expressed as nanograms of angiotensin generated per sample during the incubation period. A radioimmunoassay procedure (5) was also employed for the measurement of PRA in a number of samples and the values were similar to those obtained by the bioassay procedure (2).

*Results. Effect of SR on blood pressure.* The intravenous injection of small dose (0.6 ng) of SR into nephrectomized rats elicited a clear and prolonged (over 30 min) increase in blood pressure as shown in Fig. 1. Along with the renin-like pressor response there was a marked decrease in sensitivity of the animals to angiotensin II following the injection of SR.

*Effect of pH.* Pure SR renin was incubated with nephrectomized rat plasma at various pH values ranging from 4 to 10.5. For each sample, 6 ng of SR was added to 4 ml of plasma, the pH of each sample was adjusted to the appropriate level with 1 *N* hydrochloric acid or 1 *N* sodium hydroxide and then all samples were incubated at 37° for 1 hr. At the end of the incubation the pH of all samples was adjusted to 6 and immediately processed for the measurement of PRA. As shown in Fig. 2 the optimum pH for SR was between 8 and 8.5. The same results were obtained for both renin A and C.

The optimum pH of SR was also determined using purified renin–substrate isolated from nephrectomized rat plasma. Samples containing 80 mg of substrate and 6 ng of SR in 0.2 *M* pyrophosphate buffer of pH ranging from 4 to 10.5 were incubated for 1 hr at 37° and then the PRA was measured as above. The optimum pH was found to be the same as that found when plasma was used as substrate.

*Effect of enzyme concentration.* To samples, each containing 4 ml of nephrectomized rat plasma, various amounts of SR were added, the pH adjusted to 8 and then they were incubated for 1 hr at 37°. The PRA levels were measured by the bioassay method (2). The values obtained under these conditions indicate that the velocity of angiotensin formation was directly proportional to the enzyme concentration as shown in Fig. 3. Similar results were obtained with either renin A or C and when purified rat renin–substrate was used.

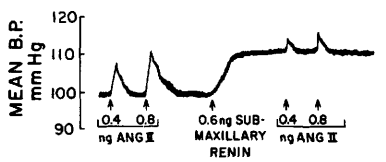


FIG. 1. Typical response of the blood pressure of bilaterally nephrectomized rats to the intravenous injection of angiotensin and SR. There is a sustained pressor response and a marked decrease in sensitivity to angiotensin following the injection of SR.

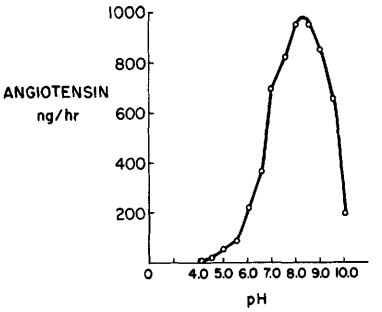


FIG. 2. Effect of pH on the rate of angiotensin generation from rat plasma renin–substrate by pure mouse SR. All samples were incubated at 37° for 1 hr.

*Effect of substrate concentration.* One ng of SR was incubated at pH 8 with various concentrations of nephrectomized rat plasma for 1 hr and the PRA values obtained for each amount of renin–substrate used, are shown in Fig. 4. The results indicate that under these conditions the rate of angiotensin formation over the range tested is proportional to the substrate concentration. Similar results were obtained when purified rat plasma renin substrate was used.

*Effect of incubation period.* An amount of 6 ng of SR was added to each of 4 ml nephrectomized plasma samples and the pH adjusted to 8. The samples were then incubated at different time intervals at 37° and the amount of angiotensin generated was measured as above (Fig. 5). The data indicate that under these conditions there is a linear time-dependence of angiotensin formation by the action of SR on renin substrate during the 2 hr incubation period.

*Reaction of SR with plasma renin substrate from other species.* PRA-free plasma from nephrectomized sheep, dog, hog, and rabbit was also used to determine whether pure SR will react with renin–substrate from these species to generate angiotensin measured as PRA. All samples were run in triplicate. When 6 ng of SR

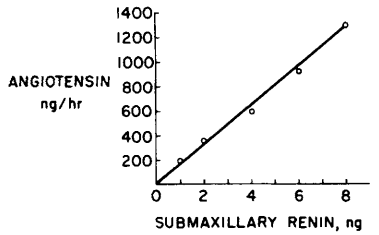


FIG. 3. Relationship between SR concentration and the rate of angiotensin formation from renin–substrate. All incubations were carried out at 37° and pH 8.0 for 1 hr.

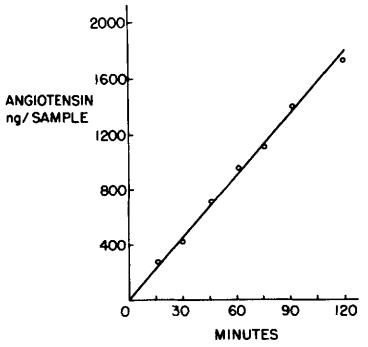


FIG. 5. Effect of duration of incubation on angiotensin liberation from renin–substrate by pure mouse SR. All samples were incubated at 37° and pH 8.0.

was incubated at pH 8.0 for 1 hr with 4 ml of plasma from these species, angiotensin was generated in all instances but to a different extent (rat 916, sheep 475, dog 124, hog 40, and rabbit 10 ng).

*Discussion.* The presence of renin-like enzymes in tissues other than the kidney has previously been demonstrated (6–9). Among these tissues the submaxillary glands of the adult male mouse contain the highest concentration of renin-like activity. Attempts to purify renin in the past have encountered great difficulties primarily due to its instability and its presence in small amount in the kidney (10, 11). Recently we have purified renin from submaxillary glands of mice (1) and have obtained it in stable and pure form. The availability of such pure SR enabled us to study its physiologic and pharmacologic characteristics.

The intravenous administration of less than 1 ng of SR into nephrectomized, anesthetized rats elicited a sustained rise in mean blood pressure which was indistinguishable from that of renal renin, and it decreased the sensitivity of the animal to angiotensin II. In addition, incubation of SR with rat renin substrate generated a material which upon injection into similarly prepared rats gave a pressor response identical to that of angiotensin. As described in our previous studies this SR hydrolyzed the synthetic tetradecapeptide renin substrate to yield a tetrapeptide and angiotensin I (1). The product of the reaction between pure SR and rat renin–substrate was also found to be immunologically identical to angiotensin I as demonstrated by a radioimmunoassay procedure (5). These findings suggest that SR is biologically similar to renin of renal origin.

The methods employed for the estimation of renin levels utilize as substrate, either whole plasma or purified renin–substrate. Most of these methods are usually based on the assumption that the pH optimum of the reaction between renal renin and renin substrate is between 5.5 and 6.0. Recently Gould *et al.* (12) reported that the optimum pH for the reaction of human renin and hog substrate is 7.5–8.5. In our studies utilizing pure mouse SR and either whole plasma or purified rat substrate the optimum pH of the reaction was found to be between 8 and 8.5. Although the previously reported optimum pH of renal renin varies with the species used as renin and/or substrate source, the optimum pH of the action of pure SR with renin–substrate found in our studies is considerably higher than what is generally believed to be for renal renin.

Some of the kinetic properties of the mouse SR are also reported. Under appropriate conditions the rate of angiotensin formation was directly proportional to the amount of enzyme, time of incubation and substrate concentration. Further, this SR does not appear to be species specific since it liberates angiotensin from plasma of a variety of animals but to a different

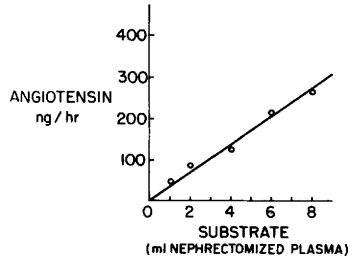


FIG. 4. Effect of substrate concentration on the rate of angiotensin formation by the action of pure mouse SR. All samples were incubated for 1 hr at 37° and pH 8.0.

extent. Whether this is due to difference in affinity between renin and substrates or due to difference in the rate of the angiotensin formation is not known.

*Summary.* The effect of the recently obtained pure submaxillary gland renin on blood pressure and its action on rat plasma renin substrate to generate angiotensin were studied. Administration of subnanogram quantities of this renin to nephrectomized rats caused a sustained rise of blood pressure and decrease in sensitivity of the animal to angiotensin. The optimum pH of its reaction with substrate was between 8 and 8.5 and under appropriate conditions the rate of angiotensin generation was proportional to the amount of enzyme, substrate concentration, and time of incubation. The enzyme liberated angiotensin from plasma renin substrate of a number of species.

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