Specificity of the Renin-Like Enzyme of Rabbit Uterus

By JAMES W. RYAN

Department of Medicine, University of Miami School of Medicine and Division of Cardiovascular Diseases,
Howard Hughes Medical Institute, Miami, Fla. 33136, U.S.A.

(Received 23 October 1969)

The pregnant uterus of the rabbit contains an enzyme virtually indistinguishable from renin of kidney (Gross, Schaechtelin, Ziegler & Berger, 1964; Bing & Faarup, 1966; Ferris, Gorden & Mulrow, 1967; Ryan & Ferris, 1967). The enzyme reacts with plasma, obtained from rabbits 48 h after bilateral nephrectomy, to produce a substance like angiotensin I. This reaction product raises mean arterial blood pressure, withstands heating at 100°C for 5 min and is inactivated by trypsin, chymotrypsin and carboxypeptidase A. On counter-current distribution the substance is partitioned like angiotensin I and is clearly distinguished from angiotensin II. Once formed, the reaction product is not inactivated during prolonged incubation with the uterine enzyme. This has been taken as evidence that the uterine enzyme, like renin, catalyses the hydrolysis of renin substrate to form angiotensin I and does not hydrolyse angiotensin I itself.

The present study was begun to determine the specificity of the uterine enzyme by using the synthetic tetradecapeptide renin substrate Asp₍₁₎-Arg₍₂₎-Val₍₃₎-Tyr₍₄₎-Ile₍₅₎-His₍₆₎-Pro₍₇₎-Phe₍₈₎-His₍₉₎-Leu₍₁₀₎-Leu₍₁₁₎-Val₍₁₂₎-Tyr₍₁₃₎-Ser₍₁₄₎ (Skeggs, Kahn, Lentz & Shumway, 1957; Skeggs, Lentz, Kahn & Shumway, 1958). This substrate was synthesized by the solid-phase technique (Merrifield, 1964; Stewart & Young, 1969) by Schwarz Bio-Research Co., Orangeburg, N.Y., U.S.A. The substrate migrated as a single spot, giving positive reactions with ninhydrin, Pauly and Sakaguchi reagents, on paper electrophoresis at pH 5, R_{Arg} 0.11, and pH 1.9, R_{Arg} 0.55 (Ryan, Roblero & Stewart, 1968b), and on ascending paper chromatography, R_F 0.6, with butan-1-ol-acetic acid-water (4:1:5, by vol.) on Whatman no. 1 paper.

The renin-like enzyme was prepared from pregnant rabbit uterus, obtained 27 days after mating. The uterus was separated from placenta and embryos and then frozen in a solid CO₂-acetone slurry. The uterus was thawed at room temperature, put through two more freeze-thaw cycles and then passed through a meat grinder. Water was added, 2 ml/g of wet tissue, and the suspension was stirred for 10 min. The suspension was centrifuged at 1690g for 30 min at 4°C. Its supernatant was diluted in 50 mM-sodium phosphate buffer, pH 6.0, to give

a protein concentration (biuret test) of 3 mg/ml. The resulting solution was heated at 56°C for 30 min to inactivate enzymes capable of degrading angiotensin (Ryan & Johnson, 1969). The final enzyme preparation contained 10000 indirect renin units/ml (Ryan, McKenzie & Lee, 1968a).

A 500 µg portion of the synthetic renin substrate was dissolved in 2.0 ml of enzyme solution and the final solution was incubated at 42°C for 1 h. The reaction was stopped by adding trifluoroacetic acid (final concn. 4%, v/v). This solution was extracted with 1 vol. of butan-1-ol previously saturated with 1% trifluoroacetic acid. The butanol layer was evaporated to dryness and the residue was redissolved in 0.3 ml of 6% (v/v) acetic acid. Samples of this solution were analysed by chromatography and electrophoresis.

Two spots were found after ascending chromatography of a 0.050 ml sample with butan-1-ol-acetic acid-water (4:1:5, by vol.) on Whatman no. 1 paper. The first, R_F 0.18, reacted strongly with Sakaguchi and Pauly reagents and weakly with ninhydrin. Its R_F matched that of authentic angiotensin I. The second spot, R_F 0.79, reacted with ninhydrin and Pauly reagents. Material from the first spot was eluted with 50 ml of 6% acetic acid, and the eluate was evaporated to dryness. Intravenous injection of this material into the pentolinium-treated anaesthetized rat (Peart *et al.* 1966) raised the mean arterial blood pressure. Its pressor potency was equivalent to 27 µg of angiotensin I.

Similarly, two spots were found on paper electrophoresis at pH 5 (Ryan *et al.* 1968b). The first, R_{Arg} 0.51, corresponded in terms of colour reactions and biological activity to the first spot of the chromatography system described above. The second spot, R_{Arg} 0.16, migrated and reacted with colour reagents as expected for the tetrapeptide Leu₍₁₁₎-Val₍₁₂₎-Tyr₍₁₃₎-Ser₍₁₄₎.

In a third experiment, electrophoresis at pH 5 was performed with Whatman 3MM paper loaded with 0.15 ml of the redissolved residue of the butanol extract. The area of paper corresponding to R_{Arg} 0.4-0.6 was cut out and eluted with 100 ml of 6% acetic acid. The eluate was evaporated to dryness and the residue was incubated with chymotrypsin (Skeggs, Lentz, Kahn, Shumway & Woods, 1956).

The reaction was stopped by heating the solution in a boiling-water bath for 5 min. The mixture was centrifuged at 1690g for 5 min and the supernatant was evaporated to dryness. Its residue was redissolved in 0.050 ml of 6% acetic acid, and the entire sample was applied to Whatman no. 1 paper for electrophoresis. Electrophoresis was carried out at pH 5 (Ryan *et al.* 1968b). Three spots were found: R_{Arg} 0.15, R_{Arg} 0.42 and R_{Arg} 0.7. The first reacted with Sakaguchi and Pauly reagents and corresponded to the tetrapeptide Asp₍₁₎-Arg₍₂₎-Val₍₃₎-Tyr₍₄₎. The second reacted with ninhydrin and Pauly reagents and migrated like Ile₍₅₎-His₍₆₎-Pro₍₇₎-Phe₍₈₎. The third reacted with Pauly reagent and developed a grey-brown colour with ninhydrin. Its migration matched that of authentic His₍₉₎-Leu₍₁₀₎.

These results show that the renin-like enzyme of rabbit uterus releases angiotensin I by catalysing the hydrolysis of the Leu₍₁₀₎-Leu₍₁₁₎ bond of synthetic tetradecapeptide renin substrate. Thus its specificity is identical with that of renin.

This work was supported in part by Grant no. M68176 from the Population Council. Synthetic tetradecapeptide was a gift from Schwarz BioResearch Co. (courtesy of Mr Roger Piazio).

- Bing, J. & Faarup, P. (1966). *Acta path. microbiol. scand.* **67**, 169.
- Ferris, T. F., Gorden, P. & Mulrow, P. J. (1967). *Am. J. Physiol.* **212**, 698.
- Gross, F., Schaechtelin, G., Ziegler, M. & Berger, M. (1964). *Lancet*, i, 914.
- Merrifield, R. B. (1964). *Biochemistry, Easton*, **3**, 1385.
- Pearl, W. S., Lloyd, A. M., Thatcher, G. N., Lever, A. F., Payne, N. & Stone, N. (1966). *Biochem. J.* **99**, 708.
- Ryan, J. W. & Ferris, T. F. (1967). *Biochem. J.* **105**, 16c.
- Ryan, J. W. & Johnson, D. C. (1969). *Biochim. biophys. Acta*, **191**, 386.
- Ryan, J. W., McKenzie, J. K. & Lee, M. R. (1968a). *Biochem. J.* **108**, 679.
- Ryan, J. W., Roblero, J. & Stewart, J. M. (1968b). *Biochem. J.* **110**, 795.
- Skeggs, L. T., jun., Kahn, J. R., Lentz, K. E. & Shumway, N. P. (1957). *J. exp. Med.* **106**, 439.
- Skeggs, L. T., jun., Lentz, K. E., Kahn, J. R. & Shumway, N. P. (1958). *J. exp. Med.* **108**, 283.
- Skeggs, L. T., jun., Lentz, K. E., Kahn, J. R., Shumway, N. P. & Woods, K. R. (1956). *J. exp. Med.* **104**, 193.
- Stewart, J. M. & Young, J. D. (1969). *Solid Phase Peptide Synthesis*. San Francisco: W. H. Freeman and Co.