Metabolism of [8-L-[¹⁴C]Phenylalanine]-Angiotensin I in the Pulmonary Circulation

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Angiotensin I is metabolized during a single circulation through the lungs (Ryan, Stewart, Leary & Ledingham, 1970). When [10-L-[¹⁴C]leucine]-angiotensin I is used as the substrate, the pulmonary-venous effluent contains radioactive His-Leu, one or more large (>7 residues) C-terminal homologues and a non-radioactive peptide having chromatographic, electrophoretic and biological properties like that of angiotensin II. In the present study [8-L-[¹⁴C]phenylalanine]-angiotensin I was perfused through the lungs to confirm the identification of angiotensin II as a metabolic product. [8-L-[¹⁴C]Phenylalanine]-angiotensin I was used because the radioisotopic label is in the angiotensin II moiety and because the distribution of charged residues (see Table 1) on either side of the phenylalanyl residue allows unequivocal electrophoretic separation of angiotensin II from all of the lower homologues of angiotensin I and II that contain phenylalanine.

Perfusion studies were performed by using Sprague-Dawley rats, weighing 0.2–0.3 kg, fed on a standard small-animal diet (Purina Laboratory Chow). The rats were anaesthetized by an intraperitoneal injection of chloral hydrate (300 mg/kg body wt.). Lungs were ventilated mechanically (respirator no. 680; Harvard Apparatus Co., Millis, Mass., U.S.A.), and the lungs and the heart were removed *en bloc*. The lungs were perfused *in vitro* via the pulmonary artery with Krebs & Henseleit (1932) solution aerated with O₂+CO₂ (95:5) and heated at 37°C. The effluent was collected from a cannula in the left atrium of the heart. The Krebs-Henseleit solution was pumped (pump no. 4; Process and Instruments Corp., Brooklyn, N.Y., U.S.A.) through the lungs at 6 ml/min. When the venous effluent became free of blood (2–3 min.), [8-L-[¹⁴C]phenylalanine]-angiotensin I (100 µCi/µmol; Schwarz BioResearch Inc., Orangeburg, N.Y., U.S.A.) was added to the Krebs-Henseleit solution to a concentration of 45 ng (about 0.035 nmol)/ml. The angiotensin I solution was pumped for 2–3 min, and the entire venous effluent was collected into an Erlenmeyer flask

containing 20 ml of cold 10% (w/v) trichloroacetic acid stirred constantly with a magnetic stirring device. At the end of the perfusion the lungs were minced with a razor blade and then homogenized in 200 ml of water in a Waring Blendor (4 min at high speed). A 1 ml portion was added to 10 ml of Scintisol-Complete (Isolab Inc., Akron, Ohio, U.S.A.) and the radioactivity was counted in a Unilux II liquid-scintillation counter (Nuclear-Chicago Corp., Chicago, Ill., U.S.A.). In four experiments the lungs retained less than 0.5% of the total amount of radioactivity used for perfusion. Recovery of radioactivity in the venous effluent was in the range 89–94%. The remainder was not found, but may have been absorbed by tubing of the perfusion circuit.

The perfusate was extracted with five 2-vol. portions of diethyl ether, and the aqueous layer was evaporated to dryness. The residue was dissolved in 1.1 ml of pyridine-acetic acid buffer, pH 5 (0.1 M-acetic acid adjusted to pH 5 with pyridine), and 1.0 ml of the solution was chromatographed on a Bio-Gel P-2 column (100 cm × 1.2 cm). The column was eluted with the pyridine-acetic acid buffer, and 60 2 ml fractions were collected. The column was calibrated previously to determine the elution volumes of angiotensin I, angiotensin II, Ile-His-Pro-Phe and phenylalanine.

A 100 µl sample of each fraction was added to scintillation vials containing 10 ml of a 0.4% (w/v) solution of Omnifluor (New England Nuclear Corp., Boston, Mass., U.S.A.) in toluene-ethanol (2:1, v/v). The radioactivities of the vials were counted in the liquid-scintillation counter.

Four major peaks of radioactivity were found. Their respective elution volumes (in fractions of 1 column volume) were: A, 0.45–0.52 (the known elution volume of angiotensin II); B, 0.59–0.71; C, 0.78–0.86; D, 0.89–0.95. Chromatography fractions corresponding to each peak were combined and freeze-dried. Each of the dry residues was dissolved in 0.5 ml of water, part being used to test effects on mean arterial blood pressure of the rat (Peart *et al.* 1966) and the remainder for electro-

phoresis. Electrophoresis was performed with Whatman no. 1 paper in systems buffered at pH 1.9 (formic acid-acetic acid-water, 6:13:81, by vol.), pH 5 (see pyridine-acetic acid buffer above) and pH 9 [1% (w/v) ammonium carbonate]. These are systems I, II and IV of Table 1. Electrophoresis was carried out at 22V/cm at 10–20mA for 1h. System III used Whatman CM-82 paper at pH 5 at 15V/cm at 5–10mA for 1.5h.

For reference in the following discussion, Table 1 shows the electrophoretic behaviours of angiotensin I, angiotensin II and some of the lower homologues of angiotensin II. Angiotensin I and its homologues were synthesized by the solid-phase method (Merrifield, 1964, 1969; Stewart & Young, 1969). The peptides obtained after deprotection were purified by countercurrent distribution. After purification each peptide migrated as a single ninhydrin- or Pauly-positive spot on paper electrophoresis at pH 1.9, 5 and 9.0, and could be hydrolysed (6M-hydrochloric acid) to appropriate component amino acids in whole molar proportions. Amino acid analyses were performed with a Spinco model 120C amino acid analyser.

Peak A was identified as angiotensin II on the basis of its elution from the Bio-Gel P-2 column, its ability to raise mean arterial blood pressure and its behaviour in electrophoretic systems I, II and IV (relative migrations: 0.62, 0.24 and 0.27; see Table 1). Electrophoresis in system II (pH 5.0)

proved to be the most useful single method of identification, as all of the C-terminal homologues of angiotensin I and angiotensin II having three or more amino acid residues migrate at rates much faster than that of angiotensin II. Pro-Phe and phenylalanine migrate at less than half the rate of angiotensin II. Peak A represented 15–20% of the total radioactivity of the pulmonary-venous effluent.

Peak B represented 55–63% of the total radioactivity of the venous effluent and contained two peptides. The first corresponded in terms of elution volume and electrophoretic migration to Ile-His-Pro-Phe; however, we were unable to exclude Pro-Phe-His-Leu. The second peptide of peak B corresponded to His-Pro-Phe, but we could not exclude Phe-His-Leu and Pro-Phe-His.

Peak C behaved as a dipeptide on gel chromatography and was neutral on electrophoresis at pH 5, and therefore was identified as Pro-Phe. Peak D was eluted as expected for phenylalanine and could not be distinguished from phenylalanine on electrophoresis in systems I, II and IV. Peaks C and D represent 13–18% and 5–7% respectively of the radioactivity recovered in the pulmonary-venous effluent.

These results confirm that angiotensin II is a metabolic product formed by lungs perfused with angiotensin I. In addition, the results of this study

Table 1. *Electrophoretic behaviour of angiotensin I and some of its lower homologues that contain phenylalanine*

Electrophoretic systems I, II and IV used Whatman no. 1 paper at pH 1.9, 5.0 and 9.0 respectively (see the text). System III used Whatman CM-82 paper at pH 5.0. Relative migrations refer to the standards, arginine (systems I, II and III) and picric acid (system IV).

System ...	Relative migration			
	I	II	III	IV
Phe-His-Leu	0.81	0.63	0.56	0.28
Pro-Phe-His-Leu	0.75	0.57	0.56	0.36
His-Pro-Phe-His-Leu	0.91	0.72	0.33	0.07
Ile-His-Pro-Phe-His-Leu	0.85	0.74	0.39	0.13
Tyr-Ile-His-Pro-Phe-His-Leu	0.76	0.65	0.33	0.09
Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu*	0.72	0.50	0.26	0.04
Asp-Arg-Val-Tyr-Ile-His-Pro-Phe†	0.62	0.24	—	0.27
Arg-Val-Tyr-Ile-His-Pro-Phe	0.68	0.65	0.13	0.22
Val-Tyr-Ile-His-Pro-Phe	0.53	0.51	0.40	0.39
Tyr-Ile-His-Pro-Phe	0.60	0.52	0.36	0.39
Ile-His-Pro-Phe	0.72	0.61	0.53	0.53
His-Pro-Phe	0.85	0.65	0.50	0.56
Pro-Phe	—	0.08	—	—
Phe	0.55	0.12	—	(0.08)
Arginine (standard)	1.00	1.00	1.00	—
Picric acid (standard)	—	—	—	1.00

* Angiotensin I.

† Angiotensin II.

agree with those of the previous one (Ryan *et al.* 1970) on the point that pulmonary conversion of angiotensin I into angiotensin II is not quantitative, the yield of angiotensin II being about 15–20%.

The rapid metabolism of angiotensin I, the failure of lung to retain significant quantities of angiotensin I or its metabolic products and the near-quantitative recovery of radioactivity in the venous effluent suggest that angiotensin I, like the adenine nucleotides (Smith & Ryan, 1970, 1971; Ryan & Smith, 1971) and bradykinin (Ryan, Roblero & Stewart, 1968; Ryan, Roblero, Stewart & Leary, 1971), is metabolized by enzymes on or close to the luminal surface of the pulmonary endothelium.

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