Release of a Renin-Like Enzyme from the Pregnant Uterus of the Rabbit

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(Received 25 July 1967)

Gross, Schaechtelin, Ziegler & Berger (1964) and Ferris, Gorden & Mulrow (1967) have reported that homogenates of pregnant rabbit uterus contain a renin-like enzyme. The enzyme resembles renin in terms of resistance to moderate acidification and precipitation at half-saturation with ammonium sulphate. When injected intravenously it causes a prolonged rise of mean arterial blood pressure, and when incubated with renin-substrate it releases an angiotensin-like polypeptide.

Although it remains to be determined whether the uterine enzyme is identical with renal renin, the fact that the enzyme releases an angiotensin-like polypeptide may be of first importance. As discussed by Braun-Menéndez (1956) and Peart (1965), all known pharmacological actions of renin appear to be mediated through the release of angiotensin. Renin reacts with renin-substrate to form the decapeptide, angiotensin I, and angiotensin I is hydrolysed by converting enzyme into the octapeptide, angiotensin II, before its biological effects are exerted (Skeggs, Lentz, Kahn, Shumway & Woods, 1956; Lentz, Skeggs, Woods, Kahn & Shumway, 1956; Peart, 1956; Elliott & Peart, 1957). Therefore it follows that any enzyme capable of hydrolysing renin-substrate to form angiotensin I or II under conditions that prevail *in vivo* may have pharmacological actions like renal renin.

The fact that homogenates of pregnant rabbit uterus contain a renin-like enzyme does not indicate that the enzyme behaves physiologically or pathologically like renal renin. On present evidence it can be argued that the enzyme has the specificity but may not have the opportunity of hydrolysing renin-substrate *in vivo*. The enzyme may be without effect unless released into a tissue space (e.g. vascular or lymphatic) containing renin-substrate and converting enzyme at a pH suitable for the formation of angiotensins I and II.

To determine if the uterine renin-like enzyme can be released, we have looked for renin-like activity in the effluent of the pregnant rabbit uterus perfused *in vitro*. Rabbits 28 days pregnant were anaesthetized with intravenous pentobarbitone (30 mg./kg.) and the uterus was approached through a midline incision. A uterine vein was cannulated and then perfused against the flow to outline the uterine segment drained by the vein. The identified segment was excised, cut along its lateral aspect, and the foetus and placenta were

removed. Aerated Tyrode's solution was perfused through the vein at 10 ml./min. The effluent was collected from the free edges of the uterine segment.

After a 60 min. wash-out period, the effluent was clear and faint yellow in colour. The effluent collected from an additional 5 min. perfusion was used as a control sample. Then a smooth-muscle-stimulating agent was added to the perfusion fluid. Compounds used were histamine (0.15 μ mole/ml.), [5-valine]-angiotensin II β -amide (Ciba Ltd., Basle, Switzerland), (0.05 μ mole/ml.) and noradrenaline (0.15 μ mole/ml.). One of these compounds was infused for 1 min. at a rate of 10 ml./min. The effluent appearing during the drug infusion and over the next 4 min. was collected as a single sample and called the stimulation sample. Effluent collected over the next 5 min. provided an index of the rate of decrease in concentration of the renin-like enzyme and was called the post-stimulation sample. Effluent collected over the next 15 min. was used as control for subsequent stimulation. A uterine segment could be stimulated three or four times without exhausting the store of renin-like enzyme.

Samples were dialysed in distilled water (4°) for 48 hr. to remove histamine, angiotensin and noradrenaline. Then 1 ml. of each sample was incubated with 1 ml. of rabbit renin-substrate; 0.5 ml. of soya-bean trypsin inhibitor (Sigma Chemical Co., St Louis, Mo., U.S.A.) (1 mg./ml.), 0.5 ml. of EDTA (disodium salt) (40 mM) and 0.05 ml. of 2,3-dimercaptopropan-1-ol (0.4 M). Soya-bean trypsin inhibitor and EDTA were in 0.1 M-sodium phosphate buffer, pH 6.0, containing chlorhexidine [1,6-di-(4-chlorophenyldiguanido)hexane] (0.01 %); 2,3-dimercaptopropan-1-ol was in arachis oil. The plasma of bilaterally nephrectomized rabbits was used as renin-substrate and was dialysed against distilled water (4°) for 24 hr. and then against EDTA (disodium salt) (3 mM) for 24 hr. before use. Distilled water and the EDTA solution contained chlorhexidine gluconate (0.01 %).

Prepared as described, the incubation mixtures were free of angiotensinase and converting-enzyme activity (Ryan, McKenzie & Lee, 1966). These mixtures were incubated at 42° for 10 hr. Reactions were stopped by heating in a boiling-water bath for 3 min. The angiotensin-like substance formed during incubation was assayed by using the blood-pressure response of the ganglion-blocked anaesthet-

Table 1. *Renin-like activity in the effluent of the pregnant rabbit uterus perfused in vitro*

Renin-like activity is expressed in $\mu\text{g.}$ of angiotensin released/hr./ml. or $\mu\text{g.}$ of angiotensin released/hr./mg. of protein.

Sample	Renin-like activity ($\mu\text{g.}/\text{hr.}/\text{ml.}$)	Concn. of protein ($\text{mg.}/\text{ml.}$)	Sp. activity ($\mu\text{g.}/\text{hr.}/\text{mg.}$ of protein)
Control	11.1	0.087	128
Histamine	31.6	0.244	130
Post-stimulation	15.8	0.110	144
Control	12.6	0.117	108
Angiotensin	50.5	0.245	206
Post-stimulation	25.3	0.141	179
Control	15.8	0.054	293
Noradrenaline	63.2	0.337	188
Post-stimulation	63.2	0.292	216
Control	42.1	0.142	297

ized rat (Peart *et al.* 1966). [5-Valine]-angiotensin II β -amide was the standard reference compound.

Results are shown in Table 1. Histamine, angiotensin, and noradrenaline caused a strong contraction of the uterine segment and a rapid rise in concentration of the renin-like enzyme in the effluent. Protein concentration (Warburg & Christian, 1936) responded similarly. Specific activity (renin/total protein) tended to increase with time.

Renin activity in normal rabbit plasma, assayed by a similar procedure, released angiotensin at a rate of $0.68 \mu\text{g.}/\text{mg.}$ of protein/hr. By comparison, specific activities of the uterine perfusates were 160–440 times as great. It is therefore very unlikely that these results could be accounted for by wash-out of trapped plasma.

To characterize the enzyme and its reaction product further, samples of dialysed perfusate were pooled and concentrated 15-fold by ultrafiltration. Then 1 ml. of the concentrated solution was incubated with 10 ml. of renin-substrate, 5 ml. of soya-bean trypsin inhibitor, 5 ml. of EDTA and 0.5 ml. of 2,3-dimercaptopropan-1-ol. Soya-bean trypsin inhibitor, EDTA and 2,3-dimercaptopropanol were prepared as described above. This mixture was incubated at 42° for 2 hr. The reaction was stopped by heating in a boiling-water bath for 10 min. The apparent angiotensin concentration was $500 \mu\text{g.}/\text{ml.}$ The clear supernatant collected after centrifugation was neutralized to pH 7.0 with 5N-NaOH, then ultrafiltered. The filtrate, containing 65% of the total angiotensin-like activity, was evaporated under reduced pressure to about 2 ml., then applied as the lower phase of the first tube of a ten-transfer countercurrent train. The countercurrent distribution system used was butan-1-ol (4 ml. each upper phase)/0.05 M-sodium phosphate buffer, pH 7.0 (2 ml. each lower phase) (modification of the system of Skeggs, Marsh, Kahn & Shumway, 1954). The apparent distribution coefficient of the

angiotensin-like reaction product was 2.0. In this system, the distribution coefficients of [5-isoleucine]-angiotensin I, [5-isoleucine]-angiotensin II and [5-valine]-angiotensin II are 1.8–2.2, 0.10–0.13 and 0.17–0.21 respectively. Thus the reaction product could not be either known form of angiotensin II and is probably angiotensin I.

The product purified by countercurrent distribution was completely inactivated when incubated for 2 hr. at 37° at pH 7.8 with trypsin, chymotrypsin or carboxypeptidase A.

These results show that the renin-like enzyme of pregnant rabbit uterus is released during perfusion *in vitro*, and its rate of release is increased by histamine, [5-valine]-angiotensin II β -amide and noradrenaline. The vasopressor product formed during incubation of the enzyme with renin-substrate is probably angiotensin I. The product, once formed, is not inactivated during prolonged incubation. Thus, like renal renin, the uterine enzyme appears to release but not degrade angiotensin I.

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