RENIN IN SUBCELLULAR FRACTIONS OF ENDOMETRIUM, DECIDUA, AMNION AND CHORION

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ABSTRACT

The amount of renin in subcellular fractions of amnion, chorion-decidua and placenta from term deliveries; of decidua from early pregnancy; and of endometrium has been measured. In addition the activation of the renin by trypsin in all the fractions was examined. The renin found in the fetal membranes was principally in the supernatant fraction and little was associated with the particulate fractions prepared (mitochondria/lysosomes and microsomes). The soluble renin was activated considerably by trypsin. Per gramme of tissue the placenta contained very little renin. Endometrium also contained small amounts of renin, but in this tissue the enzyme was found associated with the heavier particulate fraction. This renin was only slightly activated by trypsin. Decidua from early pregnancy contained much greater amounts of renin in both the mitochondria/lysosomes and supernatant fractions, but activation of the particulate material was markedly less than that of the supernatant. These results suggest that renin is stored in a granular form in these tissues but that this is not in a form activatable by trypsin. The role of such renin remains obscure.

Renin, an acid protease from the kidney, acts on its substrate in plasma to produce angiotensin I (A I) which in turn is converted to the vasoactive peptide angiotensin II (A II). The existence of extrarenal renin has been described in certain tissues including brain (4), submaxillary glands (1, 9) and tissues of the genital tract (13, 15), which suggests a possible local role for the renin angiotensin system at tissue level.

Renin from the submaxillary gland and from the kidney has been shown to be synthesised in an enzymatically inactive form (preprorenin) which, following the cleavage of a signal peptide, gave a prorenin which was still inactive and could not be converted to active renin by kallikrein or trypsin (8). It has been suggested that active renin is formed from prorenin inside the cell, since the storage form in the renin granules from both the submaxil-

lary gland and the kidney was the active form (12). Plasma, amniotic fluid and various tissues have been found to contain large amounts of inactive renin (7, 15) which can be activated by low pH, kallikrein or trypsin.

Certain uterine tissues have been shown to contain renin and be capable of producing active and inactive renin during culture (13, 15), and the endometrium has been reported to contain both renin and its substrate (5). The generation of A I *in vitro* by the fetal membranes indicated the presence of endogenous renin and its substrate in both chorion-decidua and amnion (3) and the presence of angiotensin-converting enzyme in chorion-decidua (16) suggested the existence of a locally functioning renin-angiotensin system in the human uterus. The function of extrarenal renin remains obscure; a local role, however, for angiotensin in the regulation of

sodium balance or modulation of prostaglandin activity may be important.

An investigation of the subcellular localisation of renin in these tissues and possible activation may lead to a better understanding of the role of this enzyme.

MATERIALS AND METHODS

Tissues

Fetal membranes (amnion and chorion-decidua) and placentae were obtained within 10 min of spontaneous delivery and samples of each washed in ice-cold sucrose (0.25 M) and weighed. Decidual tissue was obtained from curettings following termination of pregnancy between 7–12 weeks gestation, washed in sucrose and weighed. Endometrial tissue was taken at dilatation and curettage from fertile women admitted for tubal sterilisation and treated as described for decidua.

Subcellular Fractionation

Fetal membranes were homogenised in cold sucrose, using an ultraturrax, before resuspension using a hand held glass-glass homogeniser. Decidual and endometrial tissue did not require prior treatment with the ultraturrax before homogenisation. Centrifugation at 400 g for 10 min removed cell debris and nuclei and three further fractions of interest were prepared: M/L, mitochondria/lysosomes; material precipitating after centrifugation at 25,000 g for 20 min. P, microsomes; material precipitating after centrifugation at 100,000 g for 60 min. S, supernatant from final centrifugation.

The particulate fractions were washed once in sucrose by suspension using needle and syringe and further centrifugation. All the fractions were stored at -20°C for a maximum of 2 weeks prior to assay for renin concentration.

Renin Assay

Renin concentration was assessed indirectly by incubation of the sample under investigation with excess renin substrate and monitoring the rate of production of A I. Details of the method have been published (2). The results are expressed as ng A I produced per hour under conditions of the assay per g of original wet weight of tissue.

In some cases portions of the sample

(200 μl) were incubated with trypsin (3 μg) for 10 min at 25°C . Renin concentration was subsequently assayed following inhibition of the trypsin by an inhibitor from soybean.

RESULTS

The sensitivity of the renin assay was 300 $\text{pg}\cdot\text{ml}^{-1}\cdot\text{h}^{-1}$ and in the majority of fractions of tissues from pregnant women renin was detected. All the membranes examined contained active renin and the major part of this was found in the soluble supernatant fraction (Table 1). Small amounts of renin were associated with the particulate fractions studied, especially in samples of chorion-decidua. Only small quantities of renin were found in the supernatant fraction of the placenta. Generally renin concentrations in the membranes increased in fractions following activation of inactive renin by treatment with trypsin (Table 1); little or no activation, however, occurred in the placental fractions.

Endometrium and decidua were found to contain variable amounts of active and inactive renin. Table 2 shows results for fractions from endometrial curettings and these contained only small amounts of renin; most of the renin that was present, however, was associated with the fraction which contained the mitochondria and lysosomes, suggesting that in this tissue the renin is either in a granular form or is tightly bound to a particle.

Decidual tissue early in pregnancy contained at least 10-fold greater concentrations of renin per mass of tissue. The results shown in Table 3 suggest that approximately equal concentrations of renin are associated with heavy particles and the soluble fraction.

In decidual tissues, following activation of inactive renin, differences were found between the particulate and soluble fractions. In the soluble fractions the renin activity increased approximately fourfold, whereas the particulate activity increased only slightly.

DISCUSSION

In this study we have investigated the presence of renin in particulate fractions from chorion-decidua, amnion, placenta, decidua and endometrium in an attempt to demonstrate the existence of stored forms of renin. The storage of renin may be an important factor in controlling the rate of renin release from specific tissues.

Table 1 Active and Total Renin Concentrations in Subcellular Fractions of Fetal Membranes and Placentae from Full-term Deliveries

Sample	Fraction	Amnion		Chorion		Placenta	
		Active	Total	Active	Total	Active	Total
1	M/L	7.1	0.4	38.6	6.7	5.9	1.0
	P	2.4	0.0	9.8	4.3	3.1	0.5
	S	30.0	39.4	34.3	18.2	7.0	2.2
2	M/L	4.9	5.7	2.2	3.8	2.6	1.7
	P	1.9	0.5	2.2	0.0	2.6	0.6
	S	22.6	32.7	19.1	18.6	11.9	0.4
3	M/L	0.9	1.0	2.3	64.0	1.7	2.8
	P	0.8	0.0	0.8	6.3	1.0	0.3
	S	11.6	16.1	10.3	14.5	5.7	2.5
4	M/L	2.1	3.3	7.5	115.0	7.8	6.4
	P	0.0	0.6	5.1	11.4	1.2	1.0
	S	48.3	96.0	64.5	146.0	2.8	7.2
5	M/L	1.0	1.8	3.2	8.3	3.6	4.7
	P	2.8	0.8	3.4	1.5	4.3	0.9
	S	3.3	33.0	6.2	44.0	1.7	3.0
6	M/L	2.7	0.3	1.3	13.5	2.1	3.7
	P	1.2	0.3	2.3	6.8	1.9	0.5
	S	17.7	124.0	8.6	54.0	1.8	1.9

Results are expressed as ng A I produced · h⁻¹ · g⁻¹ wet weight tissue. M/L, mitochondrial/lysosomal; P, microsomal; S, supernatant fractions

Table 2 Active and Total Renin Concentrations in Subcellular Fractions of Endometrial Tissue

Sample	Day cycle	Fraction	Active	Total
1	8	M/L	16.0	22.0
		P	0.0	0.0
		S	9.6	51.0
2	12	M/L	16.0	32.0
		P	0.0	0.0
		S	0.0	21.0
3	13	M/L	43.0	60.0
		P	0.0	0.0
		S	8.6	17.0
4	22	M/L	0.0	0.0
		P	0.0	0.0
		S	0.0	0.0
5	25	M/L	14.0	23.0
		P	0.0	0.0
		S	0.0	18.0

Results are expressed as ng A I produced · h⁻¹ · g⁻¹ wet weight tissue. M/L, mitochondrial/lysosomal; P, microsomal; S, supernatant fractions

Histological evidence would support the existence of renin granules in extrarenal tissues similar to the situation in the kidney. Symonds *et al.* (14) showed that renin in human chorion was of fetal origin and using Bowie stain identified cells in the reticular layer of chorion to be the possible site of renin synthesis. Poisner *et al.* (10) using an indirect immunofluorescent technique found renin to be localised in the cytotrophoblast of chorion and later in the epithelial cells from amnion (11). In the uterus renin was identified in the interarteriolar connective tissue of the myometrium immediately beneath the endometrium, using a specific antibody to human renin (6). The finding of renin in the particulate fraction in this study suggests that renin is either stored in specific granules or is closely associated with other heavy structures in the cell.

Active and inactive renin have been described previously in homogenates of amnion, chorion-decidua, decidua and endometrium (15). The percentage of active renin in these homogenates and in the media from cultured explants of these tissues, was found to be

Table 3 *Active and Total Renin Concentrations in Subcellular Fraction of Decidual Tissue Taken in Early Pregnancy*

Sample	Fraction	Active	Total
1	M/L	140	250
	P	100	130
	S	483	716
2	M/L	317	350
	P	160	80
	S	500	1,050
3	M/L	450	483
	P	120	110
	S	600	2,000
4	M/L	1,000	1,000
	P	50	112
	S	433	1,133
5	M/L	145	195
	P	45	70
	S	180	455
6	M/L	>353	>353
	P	91	388
	S	209	>353
7	M/L	173	265
	P	0	0
	S	196	865
8	M/L	133	183
	P	0	0
	S	208	1,250
9	M/L	138	171
	P	9	12
	S	42	169
10	M/L	113	100
	P	13	31
	S	75	531
11	M/L	75	188
	P	0	0
	S	75	450
12	M/L	519	1,040
	P	11	37
	S	204	815

Results are expressed as ng A I produced $\cdot h^{-1} \cdot g^{-1}$ wet weight tissue. M/L, mitochondrial/lysosomal; P, microsomal; S, supernatant fractions

highly variable (15), making deductions as to the role of inactive renin difficult. In this study renin was detected in both the particulate fractions and the supernatant and when subjected to treatment with trypsin, a significantly larger proportion of inactive renin was

found in the supernatant. The renin associated with the particulate fraction consisted mainly of active renin which agrees with the hypothesis that renin in granules is prorenin which has undergone activation (12). The renin in the supernatant contained significantly larger amounts of inactive renin. This may be due to renin being secreted in an inactive form, or undergoing some conformational change following secretion as an active renin which renders it inactive. This may explain the reported difference between prorenin, which is inactive and not activated by treatment with trypsin, and the inactive renin in amniotic fluid and various tissues which can be activated by trypsin.

The presence of granules in these tissues reinforces the concept of a locally functioning renin-angiotensin system specific to the tissue. Further work is, however, required to elucidate its role in human reproduction.

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