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Subcellular localization of renin and kininogenase in the rat kidney

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Summary

1. The distribution of enzymatic activities was determined in subcellular fractions of rat kidney cortex homogenates after various homogenization procedures. The specific activities of kininogenase (KGA), BAEE esterase (pH 8·5), alkaline phosphatase and glucose-6-phosphatase were, on average, 3·4 times higher in the microsomal fraction than in the whole homogenate. The total amount of these activities in the microsomal fraction after gentle, ordinary and forced homogenization were about 15, 40 and 65% of total recovered activities, respectively. These results confirmed the localization of KGA in the microsomal fraction.

2. Renin activity was primarily recovered in the heavy mitochondrial fraction. When the force of the homogenization was increased some renin activity was shifted to the soluble fraction.

3. When a mixture of renin and purified urinary KGA was given intravenously to an anaesthetized rat, a hypotensive response due to the KGA was followed by a hypertensive renin response. Over a certain range of concentrations KGA and renin could be measured simultaneously. In fractions of kidney homogenates, however, KGA activity was too low to be measured by this method.

Introduction

This study deals with two enzymes found in the kidney: renin and kininogenase (KGA) which probably is identical with urinary kallikrein (Nustad, 1970a). Both renin and KGA are enzymes for which the appropriate substrates are found in plasma. The peptides formed by these enzymes from their plasma substrates have potent, in some cases opposite, biological effects (Page & Bumpus, 1961 ; Schacter, 1969).

Studies by Carvalho & Diniz (1966) on the subcellular localization of KGA in the kidney indicated a lysosomal localization of this activity. These authors also suggested that renin is present in the same particles as KGA and acid phosphatase in the kidney (Carvalho & Diniz, 1964). The lysosomal localization of KGA could not be confirmed in this laboratory (Nustad, 1970b). The purpose of the present study was to investigate the subcellular localization of renin and KGA to see whether the two enzymes are found in the same or in different subcellular compartments in the rat kidney.

Methods

Adult male albino rats of a local strain were used and food was withheld for 20 h before the experiments. Water was given *ad lib.* The rats were killed by a blow on the neck and the kidneys removed and placed in 0·25 M sucrose at 4° C. The cortex was removed with a pair of curved scissors. The tissue was weighed and then homogenized with a Dounce homogenizer as described by Wattiaux-de Conick, Rutgeerts & Wattiaux (1965). The force of the homogenization was varied by using two different pestles, diameters 1·49 cm and 1·51 cm, in a homogenizer with an inner diameter 1·52 cm, and by using three schemes for homogenization: 5 strokes; 10 strokes and 20 strokes.

The homogenate was differentially centrifuged using a Spinco model L2 ultracentrifuge and rotor No. 50, according to de Duve, Pressman, Gianetto, Wattiaux & Appelmans (1955). A nuclear (N), a heavy mitochondrial (M), a light mitochondrial (L), a microsomal (P), and a soluble fraction (S) were successively isolated. In most experiments, however, a combined nuclear-mitochondrial fraction (N+M+L) was first isolated, then the microsomal (P) and the soluble fraction (S) were isolated as above. All preparations were carried out at 4° C.

Rat urinary KGA

Rat urinary KGA was either dialysed rat urine or partially purified enzyme from rat urine. The latter preparation was obtained by ultrafiltration of rat urine and chromatography of the concentrate on a DEAE Sephadex (A-50) column. This preparation would hydrolyse (4·0 μmol *p*-toluene-sulphonyl-L-arginine-methyl ester (TAME)/min)/mg or (13·0 μmol N-α-benzoyl-L-arginine ethyl ester (BAEE)/min)/mg at pH 8·0 and 25° C (Nustad & Pierce, unpublished).

Enzymatic assays

KGA activity. The KGA activity of kidney fractions was bioassayed on the isolated rat uterus after gel filtration of the fraction on a column of Sephadex G-100. The substrate used was dog plasma treated by massive contact with glass and heated at 56° C for one hour (for details see Nustad, 1970a).

Renin. Renin activity was determined by the method of Skeggs, Kahn & Marsh (1953), on pentothal-anaesthetized rats, pretreated with pentolinium by intravenous injection in the external jugular vein. The blood pressure effect of the renin samples was measured in mmHg (1 mmHg≡1·333 mbar) through a cannula in the carotid artery; 0·05 ml was injected.

Other enzymes. Glutamic dehydrogenase was assayed according to Beaufay, Bendall, Baudhuin & de Duve (1959), alkaline phosphatase by the method of Bonting, Pollack, Muehrcke & Kark (1960), acid phosphatase was measured in presence of 0·1% Triton X-100 by the method of Wattiaux & de Duve (1956), glucose-6-phosphatase by the method of de Duve *et al.* (1955), BAEE esterase at pH 8·5 by the method of Roberts (1958, 1960) and proteins by the method of Lowry, Rosebrough, Farr & Randall (1951) with bovine serum albumin as reference standard.

Units of enzymatic activities. One unit of KGA activity is defined as the amount of enzyme causing the formation of kinins equivalent to 1 μg of bradykinin in 1 min in the assay conditions. Bradykinin, BRS 640, was used as standard (Sandoz,

Basle, Switzerland). One rat renin unit is equivalent to 1/40 Goldblatt dog unit of renin. Goldblatt renin, prepared from hog kidney, was used as standard (NBC, Cleveland, U.S.A.). For glutamic dehydrogenase, alkaline phosphatase, acid phosphatase, glucose-6-phosphate, and BAEE esterase, one unit is defined as the amount of enzyme which caused the decomposition of 1 μmol of substrate per min in standard assay conditions.

Results

Evaluation of the bioassays

Since kinins and angiotensins both influence the blood pressure, it is possible that the KGA present in the samples to be assayed for renin activity could interfere with the renin assay. To test this possibility, purified rat urinary KGA was added to renin standard. In these experiments either an increasing amount of KGA was added to a fixed amount of renin or an increasing amount of renin was added to a fixed amount of KGA. The results show that the hypotensive KGA effect does not interfere with the hypertensive renin effect as measured by the rat method. KGA is present in kidney tissue in amounts which are too low to interfere with the pressor response of renin. A significant error in the renin estimation was observed only when a very high level of KGA was added to the renin standard.

The specificity of the KGA assay has been evaluated previously (Nustad, 1970a).

Shift in the distribution of activities between subcellular fractions after increasing the force of the homogenization

Determination of KGA activity after differential centrifugation of kidney cortex homogenates showed that the microsomal fraction (P) contained more KGA per unit protein than the other subcellular fractions (Nustad, 1970b). Homogenates prepared by various homogenization procedures were therefore separated into a nuclear-mitochondrial (N+M+L), a microsomal (P), and a soluble fraction (S) and assayed for the various activities. The number of fractions was kept small in order to make the KGA assay, which includes gel filtration of the fractions, manageable.

When the force of homogenization was increased, one group of enzymes—KGA, BAEE esterase, glucose-6-phosphatase, and alkaline phosphatase—showed a marked, parallel shift from the nuclear mitochondrial fraction (N+M+L) to the microsomal fraction (P) (Table 1). Renin, and to some extent acid phosphatase, was not shifted to the microsomal fraction (P) but to the soluble fraction (S) (Table 1).

Subcellular localization of renin

Renin activity was found only in the heavy mitochondrial fraction (M) and the nuclear fraction (N). The distribution of renin activity after gentle homogenization is shown in Fig. 1 in the way suggested by de Duve *et al.* (1955). The distribution of reference enzymes of lysosomes: acid phosphatase, endoplasmic reticulum membranes: glucose-6-phosphatase, and mitochondria: glutamic dehydrogenase, are also shown. When ordinary homogenization was used fewer protein and enzymatic activities were recovered in the nuclear fraction (N). The specific activity of glucose-6-phosphatase was in this case highest in the microsomal fraction (P).

TABLE 1. *Shift of activities from the N+M+L to the P or the S fractions by increasing the homogenization force*

Activities	Absolute values in N+M+L+P+S homogenization			% recovered activity in P homogenization			% recovered activity in S homogenization		
	Gentle	Ord.	Forced	Gentle	Ord.	Forced	Gentle	Ord.	Forced
Kininogenase (KGA)	7·3	5·2	7·2	18·8	33·8	66·5	5·7	7·7	7·0
BAEE esterase	0·48	0·58	0·48	17·2	36·2	60·5	0	9·0	12·0
Glucose-6-phosphatase	17·4	19·5	18·4	14·9	43·2	61·5	0·8	2·2	2·8
Alkaline phosphatase	93·2	74·0	57·6	13·2	43·2	66·5	2·9	2·9	1·2
Glutamic acid dehydrogenase	0·89	1·2		1·7	2·6				
Renin	39·0	20·0	50·0	0	0	0	0	0	30·0
Acid phosphatase	5·8	5·9	8·2	11·9	16·3	16·8	12·2	14·4	29·2
Proteins	209·0	178·0	217·0	5·7	9·3	18·5	24·0	27·0	38·4

Absolute values are given in units/g wet weight of kidney cortex tissue for enzymes, and in mg/g wet weight for proteins. N, Nuclear; M, heavy mitochondrial; L, light mitochondrial; P, microsomal; S, soluble fraction.

No significant change was observed in the distribution of renin activity. Glutamic dehydrogenase, acid phosphatase and glucose-6-phosphatase showed the same subcellular distribution as reported earlier for these activities when ordinary homogenization was used, indicating proper homogenization (Nustad, 1970b).

Discussion

Kinins and angiotensins both cause contraction of the isolated rat uterus but have opposite effects on the blood pressure when injected intravenously in test animals (Schacter, 1969 ; Page & Bumpus, 1961). Renin and the kidney KGA studied have about the same molecular dimension, as determined by gel filtration (Kemp & Rubin, 1964 ; Nustad, 1970a). Renin would not therefore be separated from KGA by the gel filtration procedure used in this study. Earlier data suggested that, in the assay conditions described, KGA and not renin activity was measured in the rat uterus assay (Nustad, 1970a). The evidence included the facts that the kidney KGA had the following kallikrein-like properties in common with the urinary KGA: alkaline pH optimum, inhibition by aprotinin (Trasylol, Bayer, Leverkusen, Germany), and inactivation of the rat uterus contracting activity by carboxypeptidase B. Renin has an acidic pH optimum (Pickens, Bumpus, Lloyd, Smeby & Page, 1965), is not inhibited by aprotinin (Chiang, Erdös, Miwa, Tague & Coalson, 1968), and angiotensin II is not inactivated by carboxypeptidase B

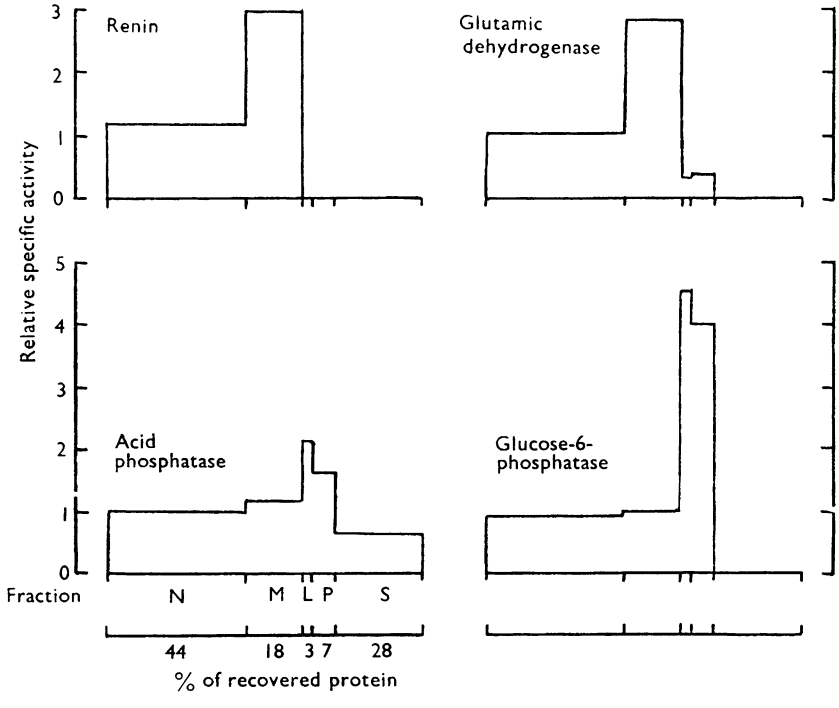

FIG. 1. Distribution pattern of enzymatic activities in subcellular fraction of kidney cortex homogenate prepared by gentle homogenization. Ordinate: relative specific activity of fractions (% of total recovered activity/% of total recovered protein). Abscissa: relative protein content of fractions. N, Nuclear fraction ; M, heavy mitochondrial fraction ; L, light mitochondrial fraction ; P, microsomal fraction ; S, soluble fraction. Total recovered activities in units/g wet weight for enzymes and in mg/g wet weight for protein are: renin 39, acid phosphatase 5·8, glutamic dehydrogenase 0·89, and glucose-6-phosphatase 17·4.

(Chiang *et al.*, 1968). No interference between renin and KGA was observed in the renin assay. The delayed appearance of the renin response in the renin assay might be explained by the time needed for angiotensin I to reach the pulmonary circulation and be converted to the highly hypertensive peptide angiotensin II (Ng & Vane, 1968). Therefore, the renin assay enabled renin and KGA to be measured simultaneously in the same sample. However, no hypotensive response was seen on the rat blood pressure when injecting kidney fractions which gave a marked renin response. Increasing the amount of material injected into the rat resulted in a minor hypotensive response only when a microsomal fraction (P) prepared by forced homogenization was tested. It was concluded, however, that the concentration of KGA in the kidney was generally too low to be assayed by this method. This was confirmed by the relatively large amount of KGA needed to produce a hypotensive response. The threshold dose corresponded to 15 mg wet weight of a microsomal fraction corresponding to 0·005 units of BAEE esterase. When purified urinary KGA was used, the threshold dose was 2 μg (in 50 μl), corresponding to 0·02 units of BAEE esterase.

It is likely that renin is synthesized in the kidney because cultured kidney cells have the ability to synthesize renin (Robertson, Smeby, Bumpus & Page, 1966). Renin has been localized in granules in modified smooth muscle cells of the afferent glomerular arterioles (for discussion of some earlier conflicting results see Bing, Eskildsen, Faarup & Frederiksen, 1967). Differential centrifugation studies showed that the granules containing renin also were rich in acid phosphatase, suggesting the granules were lysosomes (Ogino, Matsunaga, Saito, Kira, Takayasu & Ono, 1967). The lysosomal nature of the renin granules was also supported by the electron microscope studies of Robertson *et al.* (1966) and by Fisher (1966). The acidic pH optimum of renin (Pickens *et al.*, 1965) is also a feature common to other lysosomal hydrolases (de Duve, 1959), whereas the special nature of juxtaglomerular cells granules has been shown by Gomba & Soltesz (1969).

In this study renin was localized in the heavy mitochondrial fraction (M). A localization of renin to the mitochondrial fraction was also found by Dengler & Reichel (1960), Cook & Pickering (1962). The heavy mitochondrial fraction (M) includes the " renin and acid phosphatase rich fraction " of Ogino *et al.* (1967). These authors isolated their subcellular fraction according to Shibko & Tappel (1965) whereas we have used the method of de Duve *et al.* (1955). Since the lysosomal localization of renin seemed to be well established, we did not subfractionate the heavy mitochondrial fraction (M) to confirm the localization of renin. However, the distribution of renin and acid phosphatase shown in Fig. 1 clearly demonstrates that only a special group of heavy lysosomes could carry the renin activity. The solubilization of renin that took place when the force of the homogenization was increased is consistent with a lysosomal localization of the activity because the same phenomenon was observed for acid phosphatase (see Table 1). The absence of renin from the microsomal fraction (P) was also observed by Dengler & Reichel (1960) and by Ogino *et al.* (1967). It seems possible that the neutral angiotensinase which is localized in the microsomal fraction (Matsunaga, Kira, Saito, Ogino & Takayasu, 1968) could obscure some renin activity if present in this fraction.

The KGA activity is found in the kidney cortex, but it is not known whether this enzyme is synthesized in the kidney or if it represents a transient stage in the

excretion of KGA from blood to urine (Nustad, 1970a, b). Evidence for the localization of KGA to the proximal tubular cells of the nephron has been presented (Werle & Vogel, 1960). The KGA activity was not solubilized when the force of the homogenization was increased; instead, membranes of microsomal size which retained their enzymatic activity were formed (see Table 1, and Dallner, 1963). The specific activity of KGA was about the same in the microsomal fraction (P) after gentle or forced homogenization. This makes it likely that KGA is a true and not secondarily adsorbed component of the membranes which constitute the microsomal fraction (Nustad, 1970b).

The conclusion drawn from these studies is that there was no correlation between the subcellular localization of renin and KGA. This finding is consistent with the view that renin and KGA are localized in different cells of the kidney. The only other tissue in which subcellular localization of renin and KGA has been studied is the mouse submandibular salivary gland. In the glandular tissue KGA and renin-rich particles showed similar sedimentation after differential and gradient centrifugations, suggesting that both activities could be localized in the same particle. Acid phosphatase-rich particles from the glandular tissue behaved differently during differential centrifugation from those which contained renin and KGA (Chiang *et al.*, 1968). As renin was found in the heavy mitochondrial fraction and KGA was found in the microsomal fraction, no subcellular common compartmentalization between a hypertensive factor and a hypotensive factor exists, although the two factors are present in cortex.

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