Renal glycerol metabolism and the distribution of glycerol kinase in rabbit nephron

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Glycerol and dihydroxyacetone are metabolized by rabbit kidney-cortex tubules, isolated by collagenase treatment. Half-maximal concentrations of both substrates were determined with regard to uptake rates and product formation. Maximal uptake rates were 643 and 329 $\mu\text{mol/h}$ per g of protein for dihydroxyacetone and glycerol respectively. Glucose and lactate were found as major metabolic products. Glycerol kinase, the enzyme catalysing the first step in renal glycerol and dihydroxyacetone metabolism, was measured radiochemically as described by Newsholme, Robinson & Taylor [(1967) *Biochim. Biophys. Acta* 132, 338–346] and adapted for studies of the localization of this enzyme along the different structures of rabbit nephron. The results show that glycerol kinase is located exclusively in the proximal segments, i.e. the proximal convoluted tubules and the pars recta, but is negligible in the other structures studied. The activities were close to the maximal dihydroxyacetone uptake rates measured in tubule suspensions.

Besides the liver, the kidney is the only organ that plays a major role in mammalian glycerol metabolism, accounting for up to 20% of the whole-body glycerol turnover (Lin, 1977). Other organs or tissues may utilize glycerol, but at insignificant rates. Under conditions *in vivo*, the glycerol uptake by the kidney is proportional to its serum concentration (Himms-Hagen, 1968). Likewise, Robinson & Newsholme (1969a) demonstrated that rat cortical slices take up glycerol in proportion to the glycerol concentration in the medium. On the other hand, glycerol was shown to be a precursor for renal gluconeogenesis (Krebs *et al.*, 1963). Since gluconeogenesis is located in the proximal tubule of the kidney (Guder & Schmidt, 1974; Burch *et al.*, 1978; Vandewalle *et al.*, 1981), it could be assumed that glycerol metabolism might likewise be located in this nephron structure. The fate of glycerol is not, however, limited by gluconeogenesis, so that other sites of renal glycerol metabolism were not excluded. The study of renal glycerol metabolism in defined nephron segments was therefore attractive.

The first enzymic step in glycerol metabolism is phosphorylation by glycerol kinase (EC 2.7.1.30), which forms L-glycerol 3-phosphate. This enzyme is specific for glycerol and dihydroxyacetone (Wieland & Suyter, 1957). The enzyme exhibits highest activities in liver and kidney (Wieland & Suyter,

1957; Vernon & Walker, 1970), parallel with the distribution of glycerol metabolism. Since all the subsequent metabolic steps of glycerol metabolism are not specifically related to glycerol, we have measured glycerol kinase activity in different segmental microdissected tubules from rabbit kidney to identify the precise renal site of glycerol uptake. Rabbit was chosen because all nephron segments can be dissected without major difficulties from this species (Vandewalle *et al.*, 1981). With knowledge of the distribution of glycerol kinase, the products of glycerol and dihydroxyacetone metabolism studied in mixed tubule suspensions can be attributed to the proximal tubule.

Materials and methods

Materials

Glycerol and dihydroxyacetone were of analytical grade from Merck (Darmstadt, Germany) and Serva (Heidelberg, Germany), respectively. Enzymes and coenzymes were from Boehringer (Mannheim, Germany). [^{14}C]Glycerol was purchased from NEN (Dreieich, Germany). Bovine serum albumin (fraction V) and dithioerythritol were from Serva, and Dowex AG1-X8 (200–400 mesh, acetate form) was from Bio-Rad (München, Ger-

many). All basic chemicals were of analytical grade from Merck, Darmstadt, Germany.

Metabolic studies in tubule suspensions

Isolated tubule fragments were prepared from fed rabbits (female, New Zealand) by collagenase treatment (CLS II; Worthington Corp.) as described previously (Guder, 1979). Tubule incubation and sample treatment were performed as described by Wirthensohn & Guder (1980). Glycerol, dihydroxyacetone, lactate and glucose were measured by enzymic methods in the deproteinized supernatant.

Microdissection procedure

Adult female New Zealand rabbits weighing 1.5–2.5 kg were used in these experiments. They were fed on a standard laboratory diet and had access to tap water until studied. Tubules were dissected by the method described by Vandewalle *et al.* (1981). The following segmental tubules were isolated: early proximal tubule and mid portion of proximal tubule (proximal convoluted tubule), straight portion of proximal tubule (pars recta), thin descending limbs of Henle's loop, medullary ascending limb, cortical ascending limb, distal convoluted tubule, connecting tubule, cortical collecting tubule and medullary collecting tubule. When the conditions of microdissection were optimal, long superficial proximal tubules were isolated with their glomerulus and cut into segments of various lengths for determination of intratubular variation. The effect of collagenase and freezing was tested on proximal convoluted and straight tubules dissected from the collagenase-perfused kidney and the contralateral organ treated in the same way without collagenase. Glycerol kinase activity was determined in parallel in cortical and medullary homogenates from non-perfused contralateral kidneys; these were homogenized and diluted in the assay buffer (Vandewalle *et al.*, 1981).

Glycerol kinase assay

Glycerol kinase activity was determined with a radiochemical microassay which measures radioactive glycerol 3-phosphate formed from added [^{14}C]glycerol. For this, 5 μl of Hanks medium containing the tubule was mixed with 20 μl of an incubation mixture (Newsholme *et al.*, 1967), containing (final concns.): dithioerythritol, 20 mM; NaF, 26 mM; ATP, 5 mM; MgSO_4 , 5 mM; EDTA, 1.8 mM; Tris/HCl, pH 7.5, 100 mM; and various concentrations of [^{14}C]glycerol (8 mCi/mmol). Incubation was for 1 h at 37°C in Eppendorf cups. Reaction was stopped by boiling the samples for 3 min. Then 100 μl of water was added, with vigorous mixing, and 100 μl of this mixture was applied to a column containing about 0.4 g of Dowex

AG1-X8 (200–400 mesh, acetate form) equilibrated with distilled water. Glycerol was washed from the column with a further 5 ml of water. L-Glycerol 3-phosphate was eluted with 1 ml of 0.5 M-HCl, followed by 1 ml of 1 M-HCl and 2 ml of 2 M-HCl. Finally 1 ml of the combined HCl eluates was transferred to a counting vial and mixed with 10 ml of Scintigel (Roth, Karlsruhe, Germany); radioactivity was determined in a Tri-Carb liquid-scintillation counter (Packard). In each experiment we checked that glycerol elution was complete before glycerol 3-phosphate was eluted with HCl. The recovery of [^{14}C]glycerol 3-phosphate added to the incubation mixture was 102% after elution from the column. The recoveries of mixtures of [^{14}C]glycerol with unlabelled glycerol 3-phosphate and unlabelled glycerol with [^{14}C]glycerol 3-phosphate when separated by column chromatography were 105% and 103% respectively. Blank determinations were made on samples lacking tissue in each experiment. Glycerol kinase activity was expressed as pmol of L-glycerol 3-phosphate/min per mm of tubular length, measured as described by Vandewalle *et al.* (1981). In addition, enzyme activities were related to protein content, taken from a previous publication (Vandewalle *et al.*, 1981). Results are given as means $\pm$ S.E.M. Student's *t* test was used for statistical analysis.

Results

Glycerol and dihydroxyacetone metabolism in tubule suspensions

When isolated tubules from rabbit kidney cortex were incubated with increasing amounts of glycerol and dihydroxyacetone, the measured rates of substrate uptake exhibited saturation kinetics (Fig. 1). In tubules from fed rabbits, dihydroxyacetone was taken up at twice the rate of glycerol at concentrations above 5 mM. The half-maximally effective concentration was 0.5–0.7 mM for both substrates. Lactate and glucose were identified as major products. At 1 mM-glycerol, lactate formation represented 30% and glucose below 5% of substrate uptake. Glucose was a major product with 1 mM-dihydroxyacetone (30%), whereas the relative contribution of lactate formation to the carbon balance was the same with both substrates. This balance did not change to any marked extent at other substrate concentrations. Maximal uptake rates of dihydroxyacetone and of glycerol were 643 ± 109 and $329 \pm 98 \mu\text{mol/h}$ per g of protein respectively. This would require enzyme activities of 10.7 and 5.5 $\mu\text{mol/min}$ per g of protein respectively. It therefore seemed attractive to compare these metabolic data with the activities of glycerol kinase, the enzyme catalysing the first step of glycerol and dihydroxyacetone metabolism (Lin, 1977).

Fig. 1. Substrate dependence of glycerol and dihydroxyacetone metabolism in kidney-cortex tubules from fed rabbits

Kidney-cortex tubules were prepared as described by Guder (1979) and incubated over 60 min with increasing concentrations of glycerol (a) and dihydroxyacetone (b) as indicated. Metabolic changes were calculated from the differences between 0 and 60 min incubation. Values represent means $\pm$ S.E.M. for three separate experiments performed in duplicate. O, Substrate uptake, $\blacktriangle$, lactate formation, $\bullet$, glucose formation (as C_3 units). Lactate formation is presented on top of glucose formation to show their relative contributions to glycerol and dihydroxyacetone metabolism.

Glycerol kinase assay

Glycerol kinase activity has been measured by spectrophotometric (Wiand & Suyter, 1957), fluorimetric (H. B. Burch, personal communication) and radiochemical procedures (Newsholme *et al.*, 1967). For our purposes, the principle used by Newsholme *et al.* (1967) seemed most appropriate, since it possesses the sensitivity needed to measure pmol amounts of the reaction product. The filter

Fig. 2. Substrate-dependence of glycerol kinase activity in micro-dissected segments of rabbit nephron

Convoluted ($\bullet$) and straight ($\circ$) portions of proximal tubules were incubated over 60 min with increasing amounts of glycerol. Each point represents the mean $\pm$ S.E.M. for five segments dissected from the same kidney.

technique used by Newsholme *et al.* (1967) was replaced by ion-exchange chromatography in order to decrease the blank and increase sensitivity.

To obtain optimal assay conditions, the concentrations of medium constituents were re-evaluated for the present assay with rabbit cortex homogenates and isolated tubule segments. The optimal final concentration of MgSO_4 and ATP was 5 mM. In our hands NaF seemed to be unnecessary, but was always included. Glycerol kinase activity determined in homogenates showed no differences between pH 7 and 8.5; pH 7.5 was chosen for incubation, as suggested by Newsholme *et al.* (1967). In contrast with Robinson & Newsholme (1969b), we did not find any inhibition of glycerol kinase activity after inclusion of 1 mM unlabelled glycerol 3-phosphate. Likewise, this addition did not increase the amount of label in the glycerol 3-phosphate fraction. The influence of glycerol concentration, as well as time- and tissue-dependence, were tested in proximal convoluted and straight tubules. As shown in Fig. 2, glycerol kinase was saturated at 0.5 mM-glycerol in proximal convoluted tubules. In pars recta segments, dissected from another kidney, a similar dose-response curve was obtained, but glycerol kinase activity continued to rise slightly up to 2.2 mM-glycerol.

The apparent K_m was found to be 0.125 mM-glycerol in proximal convoluted tubule and 0.3 mM in the pars recta segments. In the following standard assays all tubule segments were tested at a glycerol concentration of 1 mM. Under these assay conditions, glycerol kinase activity was linear over 90 min (Fig. 3a) in both segments of the proximal tubule dissected from the same kidney. Consequently, an incubation period of 60 min was chosen for the final assay. Measurements of glycerol kinase activity as a function of tubule length are depicted in Fig. 3(b). In

Fig. 3. Effect of incubation time and tubular length on glycerol kinase activity in microdissected segments of rabbit nephron

Midportions of proximal convoluted tubules (●), straight portions (○) and medullary collecting tubules (■) were dissected from rabbit kidney, and glycerol kinase was measured as described in the Materials and methods section. In the time-dependence study (a), each point represents the mean $\pm$ S.E.M. for five segments. Each point in the tissue-dependence study (b) represents one sample, being one to three dissected segments of the same structure.

Table 1. Effect of collagenase treatment and freezing on glycerol kinase activity in proximal convoluted and straight tubule from rabbit kidney

Tubules were dissected from kidneys of the same animals; one was perfused with collagenase as described in the Materials and methods section. The contralateral kidney was treated in the same way, but without collagenase. Glycerol kinase in dissected tubules was either measured directly or after freezing on solid CO_2 as described for hexokinase (Vandewalle *et al.*, 1981). Results are means $\pm$ S.E.M., with the numbers of segments tested given in parentheses. Statistical significance was tested with the Student's *t* test. *Collagenase effect significant, $P < 0.025$.

Treatment	Glycerol kinase activity (pmol/min per mm)	
	Proximal convoluted tubule	Proximal straight tubule
None	1.738 $\pm$ 0.267 (10)	1.982 $\pm$ 0.315 (10)
Collagenase	1.185 $\pm$ 0.171 (10)	1.237 $\pm$ 0.381 (8)
Freezing	4.433 $\pm$ 0.547 (7)	2.940 $\pm$ 0.259 (9)
Collagenase + freezing	3.372 $\pm$ 0.222 (10)	1.945 $\pm$ 0.338* (10)

the range studied (0.3–5 mm), a good correlation was found in proximal segments, whereas very little activity was detectable in medullary collecting tubule segments.

Effect of collagenase and freezing

In order to dissect defined structures of the distal nephron, collagenase pretreatment was needed (Vandewalle *et al.*, 1981). Freezing was used to liberate intracellular enzyme before measurements. Therefore we tested the effect of collagenase treatment and freezing on glycerol kinase activities in proximal convoluted and straight tubules. As shown in Table 1, freezing on solid CO_2 increased glycerol kinase activity nearly 3-fold in proximal convoluted tubules, but only 50% in the pars recta, when

compared with activity in non-frozen tubules. Collagenase treatment decreased glycerol kinase in both segments studied. After freezing, this decrease was 24% in proximal convoluted tubule (not significant, owing to large variation) and 34% in pars recta ($P < 0.025$). In spite of this effect of collagenase, distal structures could not be separated without this treatment. In the following study all structures were therefore separated from collagenase-treated kidneys.

Glycerol kinase distribution along the nephron

Table 2 shows the distribution of glycerol kinase activity along the different microdissected tubular segments. When expressed per mm length as well as per μg of protein, the highest activities were found in

Table 2. *Distribution of glycerol kinase activity along the rabbit nephron*

Glycerol kinase activity was measured in single dissected nephron segments after collagenase treatment and freezing as described in the Materials and methods section. Activity is expressed as pmol/min per mm of tubular length and as pmol/min per μg of protein calculated from the protein values measured by Vandewalle *et al.* (1981). Results are means $\pm$ S.E.M., with the numbers of segments dissected from nine different kidneys given in parentheses.

Segment	Glycerol kinase activity	
	(pmol/min per mm)	(pmol/min per μg of protein)
Early proximal convoluted tubule	3.012 ± 0.261 (19)	13.08
Mid portion of the proximal convoluted tubule	3.146 ± 0.152 (28)	13.69
Pars recta	2.600 ± 0.197 (33)	11.93
Thin descending limb of Henle's loop	0.086 ± 0.046 (24)	2.11
Medullary ascending limb	0.125 ± 0.038 (8)	0.92
Cortical ascending limb	0.074 ± 0.022 (20)	0.87
Distal convoluted tubule	0.200 ± 0.041 (19)	1.37
Connecting tubule	0.173 ± 0.047 (20)	1.31
Cortical collecting tubule	0.065 ± 0.025 (8)	0.54
Medullary collecting tubule	0.207 ± 0.031 (27)	1.62

Fig. 4. *Activity of glycerol kinase along single dissected proximal tubules of rabbit nephron*

Long superficial proximal tubules were cut into segments of different length and glycerol kinase activity was measured in each segment. Each line connects the results obtained with segments from one single nephron dissected from seven different kidneys.

the proximal tubule. No difference was found between the first 1 mm ($13.2 \text{ pmol/min per } \mu\text{g}$ of protein) and the mid-portion of the proximal convoluted tubule ($13.95 \text{ pmol/min per } \mu\text{g}$). Thereafter, glycerol kinase decreased by 15% ($P < 0.05$) towards the pars recta ($11.93 \text{ pmol/min per } \mu\text{g}$ of protein). In all following segments of the nephron only very low activities were found, ranging between 0.55 and $2.16 \text{ pmol/min per } \mu\text{g}$ of protein.

In order to study whether glycerol kinase changes along single proximal tubules, enzyme activity was measured at different levels of 11 proximal convoluted tubules. As shown in Fig. 4, no change in enzyme activity could be detected up to 5 mm after the glomerulus. The variation of intratubular activities was 16.2% in 11 single proximal tubules. When

tubules dissected from different kidneys were examined, glycerol kinase activity likewise did not differ significantly. Intertubular variation was 18.3%. Thus the proximal tubules seem rather homogeneous with respect to glycerol kinase activity.

Homogenates

For comparison, glycerol kinase activity was measured in cortical and medullary homogenates of eight contralateral non-perfused kidneys. Kidney cortex exhibited an enzyme activity of $9.61 \pm 0.46 \text{ pmol/min per } \mu\text{g}$ of protein; the activity in the medulla was $3.09 \pm 0.51 \text{ pmol/min per } \mu\text{g}$ of protein. The fact that relatively high activity was found in medullary homogenates could be related to the presence of proximal straight tubules in the outer medulla of this species and possibly also to a slight contamination with remaining cortical tissue.

Discussion

The present results on glycerol and dihydroxyacetone metabolism in isolated tubules from rabbit kidney only partially confirm findings with rat kidney-cortex slices. Thus Robinson & Newsholme (1969a) and Krebs & Lund (1966) found glucose to be a major product of renal glycerol metabolism. Although this was confirmed in isolated rat kidney-cortex tubules (glucose formation represented 64% and 45% of dihydroxyacetone and glycerol metabolism, respectively, in tubules from starved rats; W. G. Guder & G. Wirthensohn, unpublished work), glucose was a minor product of glycerol and dihydroxyacetone metabolism in fed rabbits (Fig. 1). This metabolic pathway needs two gluconeogenic enzymes, which have been found to be localized in

the proximal tubule of the rat and rabbit nephron (Horster & Schmidt, 1978; for review see Guder & Ross, 1981). Therefore glucose formation from glycerol can be attributed to the proximal tubule.

Lactate formation, on the other hand, needs pyruvate kinase. The activity of this enzyme is several times higher in the pars recta than in the convoluted proximal tubule segment of rat and rabbit nephron (Guder & Ross, 1981; Horster & Schmidt, 1978). Therefore this structure is likely to be a site of lactate formation from glycerol and dihydroxyacetone.

With the present radiochemical procedure, glycerol kinase activities in rabbit kidney measured at 37°C were higher than those measured in rat kidney by Wieland & Suyter (1957) at 25°C (6.5 pmol/min per μ g) by a spectrophotometric procedure, and somewhat lower than those reported by Robinson & Newsholme (1969a) with the radiochemical procedure (14.7 pmol/min per μ g of protein, assuming a protein/fresh-weight ratio of 0.195). The highest activities in the proximal convoluted tubule (13.95 pmol/min per μ g of protein) may be compared with the results found by H. B. Burch (personal communication) in rat proximal convoluted tubule, by a microfluorimetric procedure. Assuming a protein/dry-weight ratio of 0.775, these workers report values equivalent to 6.66 pmol/min per μ g of protein. The fact that all assay procedures result in similar activities may permit the conclusion that the present assay measures the maximal capacity for renal glycerol phosphorylation. This is supported by the finding that the rate of dihydroxyacetone uptake in proximal-tubule suspensions (10.7 pmol/min per μ g of protein) is very close to the measured glycerol kinase activity (9.61 pmol/min per μ g of protein).

The higher rate of uptake of dihydroxyacetone than of glycerol can be explained by the reduced state of the cytosolic NAD couple, which limits the metabolism of glycerol 3-phosphate (Williamson *et al.*, 1969). Large amounts of L-glycerol 3-phosphate accumulate in the renal cortex after glycerol and dihydroxyacetone loading *in vivo* (Burch *et al.*, 1970) and after incubation of cortical tubules *in vitro* (Guder & Pürschel, 1980). Dihydroxyacetone phosphate does not accumulate to the same extent after dihydroxyacetone loading (Burch *et al.*, 1970).

Besides glucose and lactate, CO₂ can be a metabolic product of glycerol and dihydroxyacetone metabolism. The fact that dihydroxyacetone, but not glycerol, leads to an activation of pyruvate dehydrogenase in isolated tubules (Guder & Wieland, 1974) may explain the higher amount of dihydroxyacetone taken up and not accounted for in the carbon balance.

In addition, glycerol and dihydroxyacetone form the glycerol moiety of renal lipids (Wirthensohn &

Guder, 1980). In the presence of fatty acids, considerable amounts of glycerol and dihydroxyacetone are converted into triacylglycerol (Wirthensohn & Guder, 1980). In the present studies, acylglycerol-glycerol formation did not contribute significantly to glycerol and dihydroxyacetone metabolism, because of the absence of fatty acids from the medium.

Physiological implications

The present studies clearly point to the proximal tubule as the site of renal glycerol metabolism. Under physiological conditions, arterial glycerol concentrations are below the measured half-maximally effective concentration of renal glycerol uptake (Lin, 1977). This explains why glycerol uptake *in vivo* increases under conditions of higher blood glycerol concentrations. From the present data, the capacity of the kidneys to take up glycerol exceeds that needed under most physiological conditions. Since glycerol is filtered, this substrate can reach the proximal tubular cell from the luminal as well as the anti-luminal side. Apart from the possible role of glycerol as a metabolic fuel, lactate, glucose and acylglycerol-glycerol are the major metabolic products of renal glycerol metabolism. The fact that renal gluconeogenesis and glycerol metabolism are localized in the same cell type gives rise to the hypothesis that glycerol taken up in excess of renal needs may be converted into glucose and thereby conserved for the whole body. This may be useful under conditions of increased glycerol release from adipose tissue, such as starvation, diabetes mellitus and muscular exercise (Lin, 1977). Although no change in renal glycerol kinase activities was found in starvation (Robinson & Newsholme, 1969b), such conditions increase renal gluconeogenesis (for review see Guder, 1981) by induction of gluconeogenic enzymes.

In addition, the contribution of renal metabolism to whole-body glycerol turnover could be important in liver insufficiency or intoxication. In accordance with this assumption, renal gluconeogenesis was found to be increased in hepatic failure (Lupianez *et al.*, 1976).

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