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INTRACELLULAR DISTRIBUTION OF HEXOKINASE IN THE TISSUE ZONES OF RAT KIDNEY

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Summary

The high rates of aerobic glycolysis of tumor cells and brain may result from an increased binding of hexokinase (ATP: D-hexose 6-phosphotransferase, EC 2.7.1.1) to mitochondria. Renal papillary tissue also has a high rate of aerobic glycolysis. Therefore, the activity of hexokinase, in the mitochondrial and cytoplasmic fractions of the cortical, medullary and papillary regions of rat kidney were determined. There was an increasing cortico-papillary gradient for the specific activity (mol/kg protein per h) of total hexokinase. The specific activity of the cell-free whole homogenates of cortex, medulla and papilla were (n = 8): 0.85 ± 0.04; 2.09 ± 0.08; 3.76 ± 0.15, respectively. The specific activity of hexokinase in the papillary mitochondrial fraction (5.91 ± 0.40) was significantly greater ($P < 0.005$) than in the papillary cytoplasmic fraction, (3.40 ± 0.13). The selectively higher specific activity for hexokinase in the papillary mitochondrial fraction was in sharp contrast with the specific activity of cortical (0.96 ± 0.07) or medullary (2.28 ± 0.16) mitochondrial fractions, which have hexokinase specific activities which were not significantly different from those present in their respective cytoplasmic fractions. These observations suggest that the high rate of aerobic glycolysis of renal papillary tissue may be due, at least in part, to the high specific activity of hexokinase associated with the papillary mitochondrial fraction.

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Introduction

Of the three major tissue zones of the kidney, the papilla has a far higher rate of aerobic glycolysis than the medulla or cortex [1,2]. Neither the mechanism for the high rate of aerobic lactic acid production by the papilla nor its relation to renal function has been determined. Of potential importance is the fact that the papilla has a high hexokinase (ATP: D-hexose 6-phosphotransferase, EC 2.7.1.1) activity [3]. By contrast, the cortex of the kidney has a low rate of glycolysis and a low hexokinase activity. In brain [4] and tumor cells [5], both of which also have high rates of aerobic glycolysis, a major fraction of the total hexokinase activity is bound to mitochondria rather than being mainly localized to the cytoplasm, as is the case in many other tissues [6,7].

The fraction of cellular hexokinase which is bound to mitochondria may be important because the kinetic parameters of the bound and soluble forms of the enzyme have been found to vary, so that when the enzyme is bound to mitochondria it is more active [8—10]. Thus, if insulin or other factors were to regulate the binding equilibrium for hexokinase between soluble and mitochondrial fractions [11], such a phenomenon could modulate the rate of phosphorylation of glucose and the rate of glycolysis [12,13].

In order to gain a better understanding of the possible regulatory role of hexokinase in the metabolism of glucose and in the generation of energy from glucose in the three major tissue zones in kidney, we determined the intracellular distribution of hexokinase in cortex, medulla and papilla. Our observations show that the specific activity of hexokinase in the papillary mitochondrial fraction is selectively higher than the specific activity of the cortical or medullary mitochondrial fraction.

Experimental procedure

Materials. All of the enzymes and substrates used were purchased from Sigma Chemical Company, except for 2-oxoglutarate which was obtained from Boehringer, Mannheim. All other chemicals were of the highest purity commercially available.

Preparation of tissue for homogenization. Kidneys were obtained from male Sprague-Dawley rats (from Charles River Laboratories, Boston, MA), weighing 176—210 g (6—8 weeks old) or weighing 221—420 g (9—14 weeks old). Free access to Purina rat chow and water was allowed up to the time of the experiment. The rats were killed by cervical fracture; the abdomen was opened, and the kidneys removed, decapsulated, and placed in a beaker which was packed in ice.

Dissection of tissue zones was performed immediately on an ice-chilled Teflon cutting board using a cooled scalpel. The poles of the kidneys were discarded. Coronal sections of each kidney, approx. 2-mm thick, were made. Each kidney yielded 4—6 sections. The tissue zones from these 2-mm thick sections were separated by visual observation of the zonal boundaries, using a sharp pointed scalpel. Any tissue fragment which was contaminated with another tissue zone was discarded.

The cortical tissue used was that which was lateral to the arcuate vessels; the

medullary tissue was the dark red and light red tissue medial to the arcuate vessels. Only the 'white papilla' was used for 'papillary' tissue analysis; as a result, a portion of the inner stripe of the medulla and a small portion of the more lateral white papillary tissue were discarded.

Homogenization of tissues. All procedures for preparation of tissues for analysis were carried out at 4°C. The homogenizing buffer, as described by Borrebaek [14], consisted of 0.24 M sucrose/20 mM Tris-HCl/5 mM EDTA/5 mM $MgCl_2$/5 mM 2-mercaptoethanol. The pH was adjusted to 8.0 with KOH. The tissue from each kidney zone or from minced whole rat kidneys was weighed, and homogenized in 10—20 vol. homogenizing buffer. The majority of the homogenates were prepared with a Thomas Teflon pestle homogenizer (1 min at 1000 rev./min or 1—2 min at 600—2000 rev./min. The results obtained were the same as with a Tekmar Homogenizer (Model SDT-182, 15—30 s at half-maximal velocity). When analyses were performed on whole rat kidneys, both kidneys from an animal were combined for $n = 1$. Since rat papillary tissue comprises a small fraction of the total kidney weight, the papillary tissue from the kidneys of three to five matched rats were pooled; three rats yielded approx. 200 mg papillary tissue.

Aliquots of the homogenates of whole tissue were centrifuged at $850 \times g$ for 10 min to remove whole cells, tissue debris and nuclei. The resulting '$850 \times g$ supernatant' was pipetted off and an aliquot of this was further centrifuged at $12\,000 \times g$ for 15 min to obtain a 'mitochondrial' pellet fraction and a supernatant or 'cytoplasmic' fraction, for each sample. The mitochondrial fraction was not washed; it was resuspended in a measured volume of cold homogenizing buffer, the amount of buffer being approx. one-fifth of the volume of the original $850 \times g$ supernatant.

Assays. The method as described by Scheer et al. [15] was used to assay for the activities of hexokinase and gluconate-6-phosphate dehydrogenase (6-phospho-D-gluconate: $NADP^+$ 2-oxidoreductase (decarboxylating), EC 1.1.1.44). The assays were carried out at room temperature (24—25°C) within approx. 1—3 h after the fractions were separated by centrifugation. The hexokinase and gluconate-6-phosphate dehydrogenase activities were measured in the $850 \times g$ supernatants and in the subcellular fractions, but not in the original homonate, since tissue debris in the whole homogenates interfered in these two assay procedures. The activity of hexokinase/g fresh tissue was calculated from the observed specific activity of hexokinase in the $850 \times g$ supernatant. Therefore, it was assumed that the protein content of the $850 \times g$ supernatant was representative of the protein content in the whole homogenate.

A modification of the method of Schmidt [16] was used to measure the glutamate dehydrogenase (L-glutamate: $NAD(P)^+$ oxidoreductase (deaminating), EC 1.4.1.3) activity. Due to the fact that glutamic dehydrogenase activity is high in kidney tissue and also because it was necessary to use samples from turbid homogenates, the sample volume was decreased from 200 µl, as described by Schmidt, to 10 µl. The total volume of the reaction mixture was kept at 1 ml and the concentrations of the reagents were not otherwise altered. For some homogenate samples, as little as 10 µl of sample contained an excess of glutamic dehydrogenase activity; these samples were diluted appropriately with homogenizing buffer, and re-assayed. In order to release maximal glutamic

dehydrogenase activity from the mitochondrial matrix, the samples were subjected to multiple freeze-thaw cycles over a 1 week period prior to analysis. No loss of activity was found to occur in standards prepared from purified glutamic dehydrogenase which were subjected to multiple freeze-thaw cycles.

Protein was determined by the method of Lowry et al. [17]. Since the homogenizing buffer used contained known inhibitors of the method of Lowry et al. [17,18], the proteins were first precipitated with 5% (w/v) perchloric acid. The precipitates were solubilized in 1 M NaOH, by incubation overnight at room temperature. Protein standards of crystalline bovine albumin prepared in homogenizing buffer were treated similarly, as were the blanks. The method of Lowry et al. was then followed to measure protein content.

Statistical treatment. The paired *t*-test was used when comparing the three tissue zones from the same group of animals or when comparing subcellular fractions of the same tissue. Student's *t*-test was used when comparing different groups of animals. Differences between means were considered to be statistically significant when the *P* values were 0.05 or less. All values are reported as the mean ± S.E.

Results

Protein contents of the renal tissue zones

There is a progressive and significant decrease in protein content from cortex to papilla (i.e., between cortex and medulla, and between medulla and papilla) (Table I). These observations are consonant with the previously reported cortico-papillary gradient for dry weight of kidney tissue [19—23]. There is also a significantly greater ($P < 0.005$) mean protein content of the whole kidneys in the older group of rats (221—420 g, age: 9—14 weeks) than in the younger group of rats (176—210 g, age: 6—8 weeks). This higher protein content in whole kidneys of the older rats is also reflected in the significantly higher mean tissue protein content for cortex and medulla of the older rats. There was no difference, however, between the mean protein content of papillary tissue of the old and young rats (Table I). It is apparent that the significant cortico-papillary gradient for tissue protein content affects the magnitudes of the reported specific activities of the enzymes in each tissue zone. However, no significant differences in enzyme specific activities were found between the young and the old groups of rats.

Enzyme activities

When the hexokinase activity was determined in the $850 \times g$ supernatant of each tissue zone, the cortico-papillary gradient for the specific activity of hexokinase was the reverse of that for tissue protein content. Thus, the specific activity of hexokinase is highest in the rat papilla [3] and lowest ($P < 0.001$) in the cortex (Table II). The difference in hexokinase activity between each adjacent tissue zone is significant whether the activity is expressed per g tissue protein or per g wet tissue (Table II). Thus, while the protein content of each zone affects the magnitude of the specific activity of hexokinase, similar significant differences in the activities of hexokinase among the tissue zones are also apparent when expressed per g wet tissue.

This pattern of increasing specific activity of hexokinase from cortex to pap-

TABLE I

PROTEIN CONTENT AND ACTIVITIES OF GLUTAMIC DEHYDROGENASE AND GLUCONATE-6-PHOSPHATE DEHYDROGENASE IN RAT KIDNEY TISSUE ZONES

Values shown are the means ± 1 S.E. The numbers in parentheses indicate the number of experiments.

	Whole kidney	Cortex	Medulla	Papilla
Protein content (mg protein/g fresh tissue)				
176—210 g rats (age: 6—8 weeks)	131.7 ± 2.5 ** (7)	138.4 ± 1.3 *,** (10)	113.5 ± 1.3 ** (9)	75.4 ± 2.3 * (7)
221—420 g rats (age: 9—14 weeks)	168.8 ± 8.9 (4)	182.4 ± 6.1 * (5)	144.0 ± 5.6 (5)	83.3 ± 4.4 * (5)
Glutamic dehydrogenase activity in whole homogenates				
mol/kg protein per h	27.9 ± 1.8 (6)	22.7 ± 0.6 * (4)	17.7 ± 0.9 (4)	6.2 ± 0.6 * (4)
mol/kg wet weight per h	4.3 ± 0.2 (6)	4.2 ± 0.1 * (4)	2.5 ± 0.1 (4)	0.5 ± 0.1 * (4)
Gluconate-6-phosphate dehydrogenase activity in the 850 × *g* supernatant (mol/kg protein per h	1.07 ± 0.03 (6)	1.13 ± 0.06 (8)	1.42 ± 0.05 (8)	1.27 ± 0.07 (8)

* $P < 0.005$ when compared to the medulla within that group of rats (paired *t*-test).

** $P < 0.005$ when compared to the older rats (Student's *t*-test).

TABLE II

SPECIFIC ACTIVITY OF HEXOKINASE IN RAT KIDNEY TISSUE

Values are means ± 1 S.E. The numbers in parentheses indicate the number of experiments.

	Whole kidney	Cortex	Medulla	Papilla
Activity per kg wet tissue (mol/kg wet weight per h)	0.153 ± 0.014 (6)	0.142 ± 0.004 * (11)	0.271 ± 0.015 (10)	0.321 ± 0.019 ** (11)
Activity per kg protein (mol/kg protein per h)				
850 × *g* supernatant	0.97 ± 0.04 (6)	0.85 ± 0.04 * (8)	2.09 ± 0.08 (8)	3.76 ± 0.15 * (8)
Cytoplasmic fraction	0.94 ± 0.05 (6)	0.90 ± 0.04 * (8)	2.28 ± 0.16 (8)	3.40 ± 0.13 * (8)
Mitochondrial fraction	1.52 ± 0.07 (6)	0.96 ± 0.07 * (8)	2.28 ± 0.16 (8)	5.91 ± 0.40 *,*** (8)

* $P < 0.001$; when compared to medulla.
** $P < 0.05$; when compared to medulla.
*** $P < 0.005$ when compared to the activity in the cytoplasmic fraction (paired *t*-test).

TABLE III

PERCENTAGES OF THE TOTAL ACTIVITIES OF HEXOKINASE, 6-PHOSPHOGLUCONATE DEHYDROGENASE, GLUTAMIC DEHYDROGENASE AND PROTEIN IN THE MITOCHONDRIAL FRACTIONS

Values are means ± 1 S.E. The numbers in parentheses denote the number of experiments.

	Whole kidney	Cortex	Medulla	Papilla
Percent of total activity of the 850 × *g* supernatant found in the mitochondrial fraction:				
Hexokinase	33.3 ± 2.8 (6)	21.7 ± 2.2 (8)	21.2 ± 2.0 (8)	24.5 ± 1.6 (8)
Gluconate-6-phosphate dehydrogenase	6.8 ± 0.7 (6)	6.8 ± 0.7 (8)	7.5 ± 0.9 (8)	11.7 ± 1.2 (8)
Glutamic dehydrogenase	68.8 ± 5.4 (6)	70.4 ± 3.2 (5)	70.7 ± 2.8 (5)	73.9 ± 1.3 (5)
Protein	21.7 ± 2.3 (6)	19.5 ± 0.8 (8)	20.0 ± 0.7 (8)	15.9 ± 1.3 (8)
Percent recovery in precipitate plus supernatant, after centrifugation of the 850 × *g* supernatant at 12 000 × *g*:				
Hexokinase	103.9 ± 4.4 (6)	101.6 ± 4.7 (8)	106.0 ± 3.0 (8)	99.0 ± 3.5 (8)
Gluconate-6-phosphate dehydrogenase	103.0 ± 5.0 (6)	97.6 ± 2.6 (8)	107.1 ± 3.2 (8)	92.7 ± 3.3 (8)
Glutamic dehydrogenase	99.3 ± 6.4 (6)	106.7 ± 6.4 (5)	101.8 ± 6.8 (5)	98.2 ± 6.8 (5)
Protein	95.2 ± 1.4 (6)	95.3 ± 1.2 (8)	97.1 ± 1.4 (8)	98.0 ± 1.4 (8)

illa in homogenates of whole tissue is also present in the subcellular fractions of each tissue zone (Table II). In addition, there is a similar percentage (approx. 25%) of the total hexokinase activity present in the 850 × *g* supernatant which is associated with the mitochondrial fraction (Table III). However, the specific activity of hexokinase in papillary mitochondrial fraction is markedly elevated above that present in the medullary or the cortical mitochondrial fraction (Table II). This higher specific activity for hexokinase in the papillary mitochondrial fraction is the result of: (a) the total whole-tissue hexokinase activity is highest in the papilla, while (b) the protein content is lowest in the papillary mitochondrial fraction, and (c) a similar fraction of the total hexokinase present in the 850 × *g* supernatant of each zone is associated with the mitochondrial fraction.

Whilst the mitochondrial fractions obtained from the cortex and medulla have the same specific activity for hexokinase as do their respective cytoplasmic fractions, the specific activity of hexokinase in the papillary mitochondrial fraction is significantly greater ($P < 0.005$) than the specific activity of the papillary cytoplasmic fraction. As a result, the specific activity of hexokinase in the papillary cytoplasmic fraction is significantly ($P < 0.01$) decreased below that in the 850 × *g* supernatant of papillary tissue (Table II). It should be noted that, based on the hexokinase content of the 850 × *g* supernatant, the recovery of hexokinase in the cytoplasmic plus the mitochondrial fraction was essentially complete (Table III).

In order to assess the degree of purity of each subcellular fraction, the specific activities of gluconate-6-phosphate dehydrogenase, a cytosolic marker [15] and glutamate dehydrogenase, a mitochondrial marker [16] were assayed (Tables II, III). There is little data available concerning the activities of these two enzymes in each of the kidney tissue zones, particularly with respect to the papillary and medullary zones of the kidney.

The specific activity of gluconate-6-phosphate dehydrogenase in the 850 × *g* supernatant is similar in all three zones of the rat kidney (Table I). As a result, in the cortex, the activity of gluconate-6-phosphate dehydrogenase, the second enzyme of the hexose monophosphate shunt, is approx. equal to the activity of hexokinase. However, in the papilla, due to the increase in hexokinase activity, the gluconate-6-phosphate dehydrogenase activity is one-third that of the hexokinase activity.

In contrast to the similar activities of gluconate-6-phosphate dehydrogenase in each of the three renal tissue zones, there are significant decreases in glutamic dehydrogenase activities between cortex and papilla of the whole homogenates (Table I). The intrarenal distribution of glutamic dehydrogenase activity is consistent with that reported in the histochemical study of Schmidt and Dubach [24]. If one assumes that the ratio: activity of glutamic dehydrogenase/unit weight of mitochondrial protein, is constant in all tissue zones of the kidney, then these present observations (Table I) indicate that in the papilla, there is approx. one-fifth as much mitochondrial protein as is present in cortex and one-third as much as is present in medulla. Thus, our observations are consistent with previous reports [25,26] that the papilla has the lowest mitochondrial mass/unit weight of kidney tissue.

The degree of cross-contamination of the subcellular fractions was found to

be low (Table III). Less than 12% of the gluconate-6-phosphate dehydrogenase in the 850 × *g* supernatant was found in the mitochondrial fraction. Also, less than one-third of the glutamic dehydrogenase activity of the 850 × *g* supernatant was found in the cytoplasmic fractions.

Discussion

These observations show that the specific activity of hexokinase in the mitochondrial fraction of rat renal papillary tissue is greatly elevated over that in the cytoplasmic fraction. In contrast, the cortical and medullary mitochondrial fractions have essentially the same specific activities of hexokinase as do their cytoplasmic fractions. Our observations provide no definitive evidence that the hexokinase was in fact bound to the mitochondrial fraction. However, from other studies [4—14,27], it is probable that the hexokinase was bound to the mitochondria. Whether this selective association of hexokinase with the papillary mitochondrial fraction is due to the presence of a high content of a specific hexokinase-binding protein [27] in papillary mitochondria remains to be determined.

That the specific activity of hexokinase of the mitochondrial fraction is highest in the rat papilla is of importance in light of the reports that such an interaction of hexokinase with mitochondria may increase the efficiency of the phosphorylation of glucose by providing a direct link between the mitochondrial oxidative phosphorylation system and the cytosolic glycolytic pathway [5,8—13]. Indeed, the increased specific activity of hexokinase of mitochondria may also permit the formation of large amounts of glucose 6-phosphate and ADP, without inhibiting the hexokinase by the product, glucose 6-phosphate [5,7,13]. An increased rate of entry of glucose 6-phosphate into the glycolytic pathway, would then result in the rapid formation of pyruvate. The smaller mitochondrial content of the papillary tissue (Table I) limits the amount of pyruvate that can be oxidized [1] and could account for the observed accumulation of pyruvate and in this tissue region [1,28,29]. Therefore, the selective binding of hexokinase to papillary mitochondria may be the mechanism for the observed high rate of aerobic glycolysis in rat tissue. A high degree of binding of hexokinase to mitochondria has also been postulated to be the basis for the high rates of glycolysis in tumor tissue [5] and brain [11,13].

The potential significance of these observations for renal metabolism is that if the degree of binding of hexokinase to papillary mitochondria can be modulated by physiological phenomena, a change in the rate of glucose utilization by the tubular elements contained in the renal papilla may occur. For example, the papillary tissue content of lactate is increased during an osmotic diuresis in the golden hamster [28], the rat [29] and the dog [30]. This accumulation of lactate in papillary tissue occurs relatively rapidly and could reflect an increase in the degree of binding of hexokinase to mitochondria.

An increase in the degree of mitochondrial binding of hexokinase may also be mediated by insulin [8,9,11,14]. The anti-natriuresis and increase in negative free-water clearance which occur following ingestion of a high carbohydrate-content meal (but not a high fat-content meal), by fasted individuals, has been attributed to the effect of insulin on the rate of glucose utilization by the

distal nephron segments [31]. Indeed, insulin has been shown to increase both net Na^+ reabsorption 'beyond the proximal tubule', in the dog in vivo [31], and also to increase the rate of glucose decarboxylation by the isolated perfused rat kidney [32].

Overall, our observations showing a selective association of hexokinase with the papillary mitochondrial fraction, when compared with the medullary and cortical mitochondrial fractions, indicate that such a phenomenon may account for the high rate of aerobic glycolysis of renal papillary tissue.

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