RENAL METABOLISM OF GLUCOSE: ANATOMICAL SITES OF HEXOKINASE ACTIVITY IN THE RAT NEPHRON

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1. Introduction

The relationship between renal glucose transport and catabolism has been a subject of intense investigation [1]. Despite the high filtered load of glucose and active cortical synthesis of glucose, there appears to be little intrarenal catabolism. In the proximal nephron where virtually glucose is reabsorbed [2,3] glycolytic activity is low [4]. Nevertheless others have demonstrated that there is medullary glucose catabolism in vivo and in the isolated perfused kidney [5]. Since hexokinase is a key enzyme of the glycolytic sequence [6] the localization of this enzyme in various sites along the rat nephron would give insight into the relationship between glucose transport and catabolism. Utilizing a micro-dissection technique of lyophilized kidney slices [7] we were able to examine the activity of hexokinase in single portions of the rat nephron.

2. Methods

4 male Wistar rats (200 g) with food and water ad libitum were nephrectomized. Kidney tissue mounted on a holder was dropped into liquid nitrogen. Serial sections of 16 μm were cut in a cryostat. The first of two consecutive sections was lyophilized under vacuum over 12 hr, the second was stained with periodic acid-Schiff's base reagent. The stained section served as guide for the identification of the desired tubular structure in the lyophilized section. Glomeruli were dissected from the subcapsular area. Proximal convolutions were identified by their deeply red stained brush border and their localization near the glomeruli. Proximal straight portions were localized at the tip of the medullary ray. The thick ascending limbs of Henle were distinguished in the inner stripe of the outer medulla by their brownish cytoplasm. Distal convolutions were dissected from the surrounding of glomeruli. Table 1 lists the dry weights of pooled and of single samples and the amount utilized in the assay.

2.1. *Hexokinase*

Hexokinase activity was measured according to the method of Lowry et al. [6] with slight modifications in the incubation medium which contained the following reagents at final concentrations: glucose 8 mM, Tris–HCl buffer 0.1 M, pH 8.0, KH_2PO_4 10 mM, pH 8.0, $MgCl_2$ 5 mM, ATP 3.4 mM adjusted to pH 7.8, NADP 0.2 mM, bovine serum albumin 0.05%, glucose-6-phosphate dehydrogenase 2.45 μg/ml. Dry tissue was transferred into tubes at controlled room temperature and 10 μl of incubation medium were added. The samples were incubated for 30 min at 37°C and then transferred back to 0°C; 30 μl of 0.075 N NaOH were added. Excess NADP was now destroyed by heating for 15 min at 60°C; after cooling 150 μl of a 8 N NaOH/H_2O_2 mixture (3.0 ml 8 N NaOH plus 20 μl 3% H_2O_2) were added and the tubes then incubated for 60 min at 37°C. 150 μl aliquots were transferred to fluorometer tubes containing 0.8 ml of a 1 N NaOH/EDTA mixture (0.5 ml 1 N NaOH plus 0.25 ml 0.1 M EDTA into 25 ml Aqua bid.) and the fluore-

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scence was measured with a Farrand-filter fluorometer equipped with filters No. 5860 as the primary and Nos. 4308 and 3389 as secondary combinations. Blank and standards of 0.09 and 0.18 mM NADPH were treated as the samples. One unit of activity is defined as the formation of one nmol NADPH/mg dry weight/min at 37°C.

The obtained data were analyzed statistically with the student 't' test. The $p < 0.01$ level of probability was used as criterion for significance.

Table 1
Dry weight of nephron structures in nanogram used for hexokinase determination

Structure	Pooled sample	Single sample
Glomerulus	190 *	28
Proximal convolution	125	18
Proximal straight portion	120	17
Thick ascending limb of Henle	60	10
Distal convolution	85	5

* Pooled sample is used for one analysis

3. Results and discussion

Table 2 summarizes the hexokinase activities of various structures of the rat nephron. The highest activity was detected in the thick ascending limb of Henle's loop, which was followed by the distal convolution with 75%, the proximal straight portion with 13%, the glomerulus with 10% and the proximal convolution with only 6% of the highest activity value. A similar pattern of hexokinase distribution along the nephron was also obtained when the amount of formed NADPH was referred to millimeter tubular length, as shown in the second column in table 2. The differences between proximal convolution and proximal straight portion as well as between thick ascending limb of Henle and distal convolution are statistically significant ($p < 0.01$).

Perfusion studies which have shown that net glucose uptake rate by the cortex is only one third the uptake rate by the medulla [8] are in accordance with the low activity value in the proximal part of the nephron. The hexokinase activity is fifteen times higher in the thick ascending limb and ten times higher in the distal convolution compared with the proximal convolution. The distal convolution is localized in the cortex and it may be that the glucose uptake rate of the cortex is mainly supported by the distal tubule. The very low hexokinase activity in the proximal convolution does not exclude that glucose is a source of exogenous fuel of respiration by this structure. Based on the data in table 2 one can calculate that the hexokinase in the proximal convolution is as high (0.1 pmol/mm/sec) as to produce anaerobically at least 50% of ATP (0.2 pmol ATP per mm of length per sec) needed to maintain transport processes in this tubular portion (active component of Na net flux in the proximal convolution (rat) amounts 1.5 pmol/mm/sec [9]).

Earlier investigations have suggested that glucose reabsorption is mediated by phosphorylation [1]. This segment, however, revealed the lowest hexokinase activity (table 2). In the proximal convolution of the rat nephron a glucose reabsorption could be calculated of $35.06 \pm 17.6 \times 10^{-12}$ mol $\times$ mm^{-1} $\times$ min^{-1} [10].

Table 2
Localization of hexokinase activity along the nephron in rat

Site	Dry weight	mm of length
Glomerulus	12.0 ± 1.8 (5) *	–
Proximal convolution	8.2 ± 3.8 (5)	2.2 ± 1.0 **
Proximal straight portion	15.7 ± 5.0 (5)	4.0 ± 1.3
Thick ascending limb of Henle	125.3 ± 7.2 (5)	27.2 ± 1.5
Distal convolution	95.8 ± 8.8 (5)	13.0 ± 1.2

* Nanomoles converted substrates per mg dry weight of rat tissue per min ± SD.
** Picomoles converted substrates per mm of length per min ± SD.
Number of analyses in brackets.

Based on our present findings only 5% of glucose entering the cell could be phosphorylated by the hexokinase activity present. Thus, it appears that the phosphorylating step accounts for only a small fraction of the proximal glucose transport. These results are in good agreement with other studies utilizing C-labeled D-glucose which have shown that the fraction of D-glucose (3%) entering the glycolytic pathway is also within this range [11].

Recent studies have demonstrated a linear rise in glucose oxidation in the outer medulla when extracellular sodium was changed from zero to 326 mM. In addition, ouabain, a steroid inhibitor of Na-K-ATPase, completely abolished the sodium induced acceleration of glucose oxidation [12]. In the outer medulla where the thick ascending limb predominates, the source of energy of respiration for hyperosmotic sodium transport appears to be derived to a large extent from the oxidation of exogenous glucose. Our present finding of a high phosphorylating activity in the thick ascending limb supports this observation. The hexokinase in the distal tubule is high enough to metabolize the small amount of glucose entering the cell from the luminal side. The observed glucose reabsorption in the distal tubule could therefore be mediated by phosphorylation.

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