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Pathways for organic osmolyte synthesis in rabbit renal papillary tissue, a metabolic study using ^{13}C-labeled substrates

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Renal papillary collecting duct cells have been postulated to adapt their intracellular osmolality to the large changes in interstitial osmolality by changing their content of 'non-perturbing' organic osmolytes such as sorbitol and *myo*-inositol. ^{13}C-NMR was used in this study to elucidate the metabolic pathways leading to a synthesis of those compounds. Incubation of rabbit renal papillary tissue with [1-^{13}C]glucose showed label scrambling mainly into sorbitol (C-1) and lactate (C-3). This result confirms activity of aldose reductase and glycolytic enzymes in renal papillary cells. Using [3-^{13}C]alanine or [2-^{13}C]pyruvate as carbon source, ^{13}C-labeling of sorbitol and *myo*-inositol was observed, indicating that renal papillary tissue possesses, in addition, gluconeogenic activity. The latter assumption is supported by the result that in enzyme assays rabbit kidney papilla and isolated rat kidney papillary collecting duct cells show significant fructose-1,6-bisphosphatase activity.

Urine produced in the mammalian kidney can reach an osmolality which is up to ten-times higher than in renal plasma [1]. Concentration proceeds, during the passage of the tubular fluid, through medulla and papilla [2]. Thus, papillary cells are surrounded by hypertonic fluid, which contains urea and sodium chloride as the main solutes [3]. It has been postulated that in order to adjust intracellular osmolality renal papillary cells produce 'nonperturbing' organic solutes, such as sorbitol [3,4], inositol, glycerophosphorylcholine [4,5], and betaine [5]. Thus far, studies related to these organic solutes have concentrated on the determination of solute content, only a few studies are available dealing with the synthetic pathways of these compounds.

^{13}C-NMR spectroscopy using ^{13}C-enriched substrates provides a powerful tool for the investigation of metabolic pathways in cells and tissues. We, therefore, applied this technique to examine the metabolic pathways of inositol, sorbitol and glycerophosphorylcholine in rabbit renal papilla.

Papillary tissue was obtained from male white rabbits. The animals were killed by a sharp blow to the neck, the kidneys were excised and immediately perfused with a Krebs-Ringer's solution (2–4°C). After dissection, the papillary tissue was rinsed with a Ringer's solution devoid of any carbon source and was cut into small slices (thickness 1–2 mm). These slices (0.25 g) were incubated at 37°C in 10 ml Krebs-Ringer's solution, containing the ^{13}C-labeled carbon sources as

Abbreviations: PCA, perchloric acid; TCA, tricarboxylic acid.

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required. The standard Krebs-Ringer's solution was of the following composition (in mM): NaCl (128)/$NaHCO_3$ (25)/KCl (3.2)/$CaCl_2$ (2.5)/$MgSO_4$ (1.8)/KH_2PO_4 (1.8)/D-glucose (8) L-lactate (4)/L-alanine (2)/adenosine (0.5). The pH was adjusted to 7.4 by aeration with 5% CO_2/95% O_2.

For incubation with ^{13}C-labeled substrates the ^{13}C-labeled carbon source was the only substrate. At the end of the incubation period, the sample was fractionated into supernatant and cells by centrifugation at 4°C for 7 min at 3500 rpm. The cells were extracted with perchloric acid (PCA), the extract was lyophilized at 4°C and dissolved in 1.7 ml ^2H_2O.

^{13}C-NMR spectra were recorded at 100.61 MHz on a Bruker AM 400 WB spectrometer equipped with an aspect 3000 computer. Typical acquisition parameters were as follows: spectral width 21800 Hz, pulse width 12.0 μs (corresponding to a 90° flip angle), data size 16K, relaxation delay 1.7 s. Proton decoupling was carried out by a standard composite pulse sequence. The ^{13}C-NMR spectra of PCA extracts were obtained in 10-mm tubes at 5°C and FIDs (free induction decays) consisting of 20000 scans were accumulated. Chemical shifts (δ, ppm) were referenced to L-[3-^{13}C]alanine at 17.11 ppm (internal standard). Assignments were made on the basis of the chemical shift values given in the literature [6,7], or by recording ^{13}C-NMR spectra of the authentic compounds under the same conditions as the extracts and supernatants.

The integrated intensities of the glutamate and glutamine resonances were corrected for T_1 (spin-lattice relatation time) and NOE (nuclear Over-

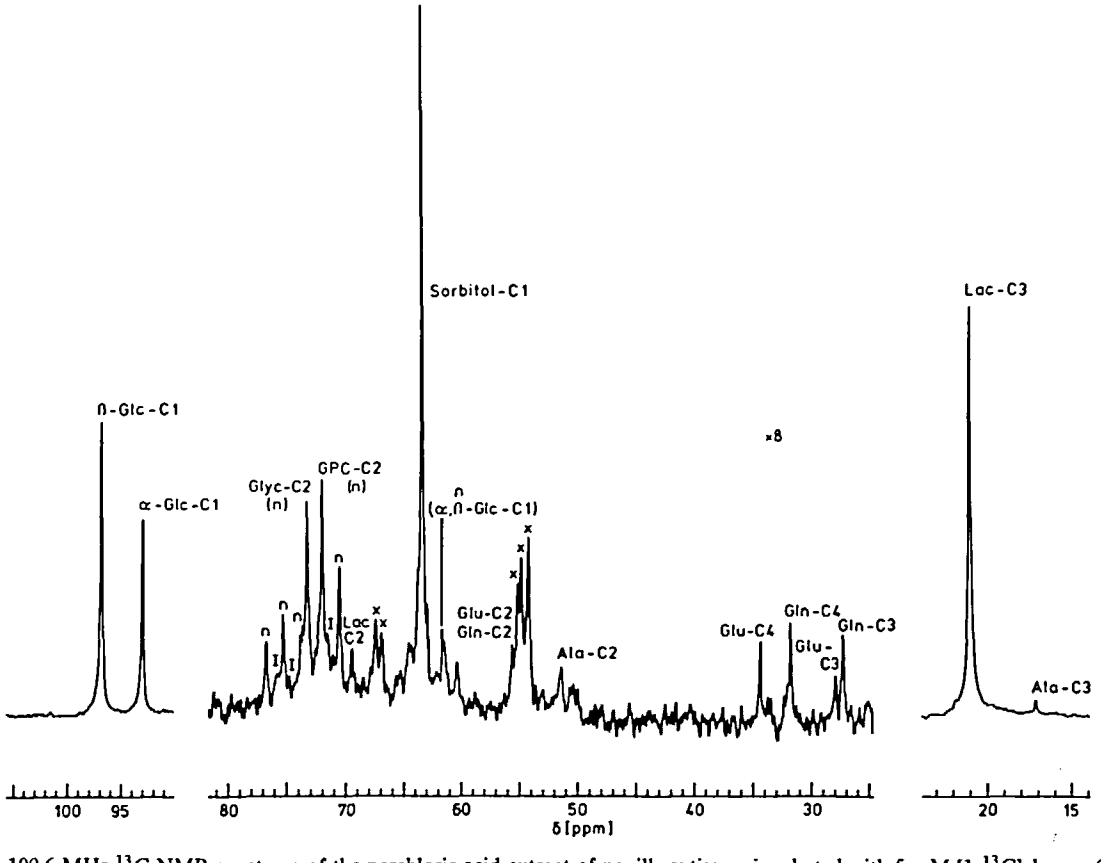

Fig. 1. 100.6 MHz ^{13}C-NMR spectrum of the perchloric acid extract of papillary tissue, incubated with 5 mM [1-^{13}C]glucose ($n = 3$) for 2 h at 37°C. *n*, natural abundance contribution of the non-labeled carbon atoms of D-[1-^{13}C]glucose; x, resonances due to choline and betaine, which mainly consist of natural abundance ^{13}C; I, inositol.

hauser effect) under extract conditions by using a 0.1 M standard solution of glutamate and glutamine.

For the estimation of the ratio between pyruvate carboxylase activity and pyruvate dehydrogenase activity [7], we compared T_1- and NOE-corrected pool sizes (G_i, G_j) for glutamate and glutamine. We define

$$[G_{i,j}] = [C_{i,j}]_{glu} + [C_{i,j}]_{gln} \quad (1)$$

with $[G_{i,j}]$, line intensities for the pool of all i or j carbon atoms of glutamate and glutamine.

All ^{13}C-labeled compounds used in this study were obtained from Merck, Sharp & Dohme Isotopes, Montreal, Canada. Sorbinil was obtained from Pfizer Central Research, Groton, CT, U.S.A.

In view of the known high aerobic glycolytic activity [8] and the presence of aldose reductase in renal papilla [3,5], we first examined the utilization of D-[1-^{13}C]glucose (5 mM) by rabbit renal papillary tissue. The ^{13}C-NMR spectrum of the corresponding perchloric acid extract is presented in Fig. 1. Four intense and various less intense resonances are shown.

The intense resonances at 96.8 and 93.6 ppm correspond to ^{13}C-labeled βC-1 and αC-1 carbon of glucose taken up by the cells. The intracellular glucose also gives rise to resonance peaks in the region of 70–77 ppm and at 61.4 ppm, due to a natural abundance of ^{13}C in the C-2–6 carbons of α,β-glucose.

The intense signal at 20.9 ppm represents carbon C-3 of lactate formed from the labeled D-glucose (C-1), via the glycolytic pathway. The intense resonance at 63.14 ppm can unambigiously be assigned to sorbitol (C-1), indicating a high aldose reductase activity in the tissue. In addition, less intense resonances, due to labeled glutamine (27.6, 33.5 and 55.4 ppm) and glutamate (27.8, 34.4 and 55.7 ppm), indicate an influx of the ^{13}C label into the TCA cycle. Furthermore,

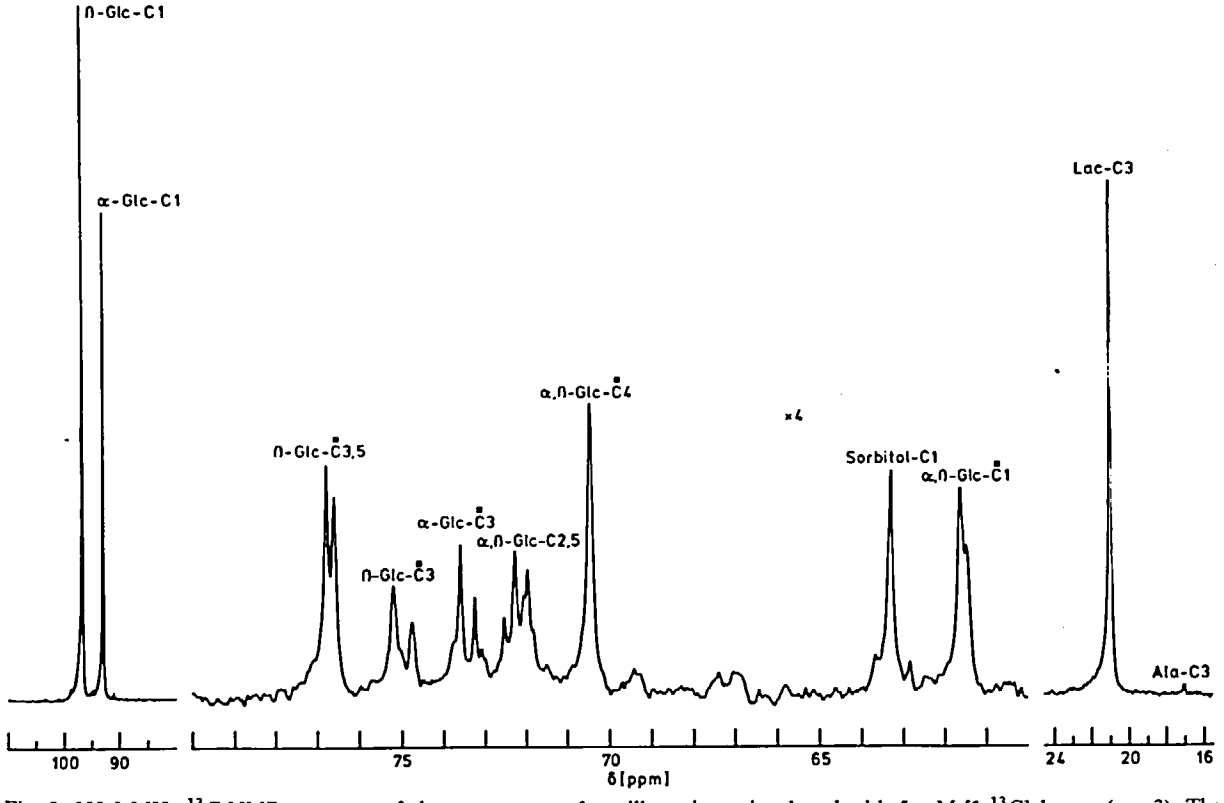

Fig. 2. 100.6 MHz ^{13}C-NMR spectrum of the supernatant of papillary tissue, incubated with 5 mM [1-^{13}C]glucose ($n = 3$). The resonances due to the natural abundance glucose are indicated with ■.

a weak but significant labeling of alanine (52.5 ppm) and of lactate (69.8 ppm) at the C-2 position was found. This labeling can be explained by the formation of C-2-labeled phospho*enol*pyruvate from C-2-enriched oxaloacetate by phospho*enol*-pyruvate carboxykinase (EC 4.1.1.32) and a subsequent formation of C-2-labeled pyruvate via pyruvate kinase.

The label exchange at the phospho*enol*pyruvate stage is also reflected in the labeling of the C-2 carbon atom of glycerol (73.12 ppm) and of the corresponding carbon atom of glycerolphosphorylcholine (71.6 ppm). Furthermore, the data of Fig. 1 indicate the presence of relatively high intracellular pools of betaine and choline. Experiments with unlabeled glucose revealed that these resonances are mainly due to the natural abundance of ^{13}C in betaine and choline.

Fig. 2 shows the ^{13}C-NMR spectrum of the corresponding supernatant. Again, the most predominant resonances belong to C-1 of α,β-glucose and to lactate (C-3). In the 61–78 ppm region, also the resonance of sorbitol (C-1) can be clearly distinguished. These results indicate that labeled lactate and labeled sorbitol leave the cells and appear in the extracellular medium.

In the presence of 1 μM sorbinil, a known inhibitor of aldose reductase [11], intracellular sorbitol synthesis as well as sorbitol release were almost completely inhibited (85%, $n = 2$).

The data shown above demonstrate qualitatively the activity of aldose reductase, glycolytic and TCA cycle enzymes in renal papillary tissue. They also suggest the possibility of gluconeogenesis in the rabbit papilla.

Further information on these metabolic pathways was obtained by incubation of papillary tissue with 5 mM L-[3-^{13}C]alanine. Alanine is converted by a desamination to [3-^{13}C]pyruvate, a main branching point of metabolism [12].

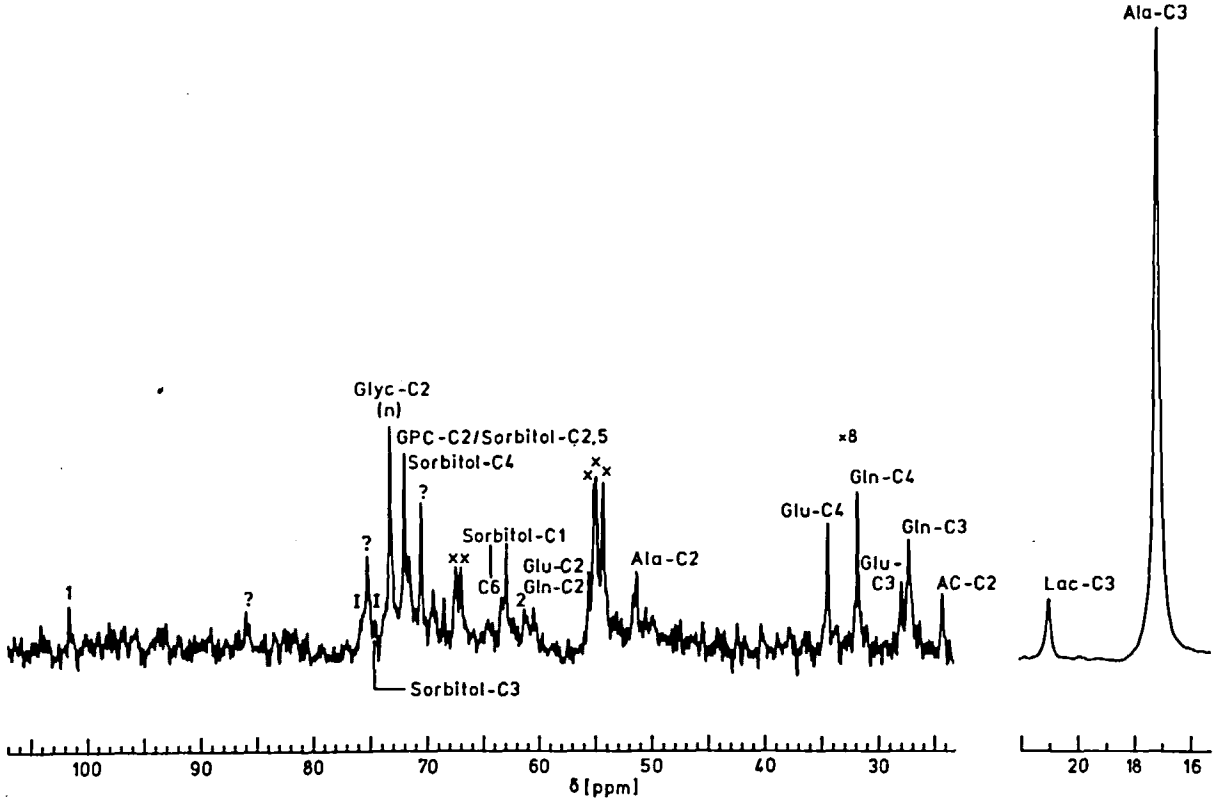

Fig. 3. 100.6 MHz ^{13}C-NMR spectrum of the perchloric acid extract of papillary tissue, incubated with 5 mM L-[3-^{13}C]alanine ($n = 2$). *n*, natural abundance contribution in partly labeled glycerol (ester); 1 and 2, C-1 and C-6, respectively, in glycogen.

The ^{13}C-NMR spectrum of the perchloric acid extract obtained from the tissue after incubation with (3-^{13}C]alanine ($n = 3$) is shown in Fig. 3. Besides, L-[3-^{13}C]alanine, resonances of [3-^{13}C]lactate (20.9 ppm), [2-^{13}C]lactate (69.8 ppm) and [2-^{13}C]alanine (53.8 ppm) and of C-2-, C-3-, and C-4-labeled glutamine and glutamate are found. The latter originate from [3 – ^{13}C]alanine entering the TCA cycle via pyruvate carboxylase and pyruvate dehydrogenase.

The labeling of the carbon atoms of glutamate and glutamine reflects the labeling in the TCA cycle intermediate, α-ketoglutarate, which, in turn, is a consequence of the influx of ^{13}C-label into the TCA cycle via pyruvate carboxylase and pyruvate dehydrogenase [7,13], as has been shown in perfused livers [7] and renal epithelial LLC-PK$_1$ cells [13]. The relative enrichment of the C-2 and C-3 glutamate carbon atoms are a consequence of pyruvate carboxylase activity, whereas a label influx via pyruvate dehydrogenase results in labeling of the C-4 carbon atom of glutamate and glutamine.

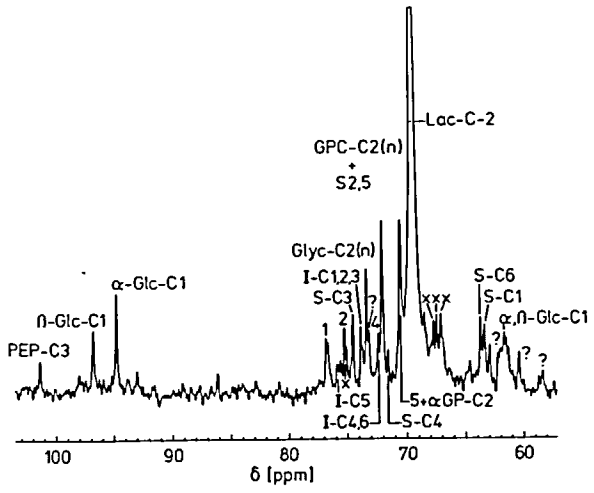

Fig. 4. The 60–110 ppm region, 100.6 MHz ^{13}C-NMR spectrum of the supernatant of papillary tissue, incubated with 5 mM [2-^{13}C]pyruvate ($n = 2$). n, natural abundance in partly labeled glycerol ester; x, resonances due to choline and betaine, which mainly consist of natural abundance ^{13}C; I, inositol; S, sorbitol; 1, β-glucose, C-3,5; 2, β-glucose, C-2; 3, α-glucose C-3; 4, α-glucose, C-2,5; 5, α,β-glucose, C-4.

Considering the pools of labeled glutamate and glutamine ($G_{i,j}$), one can estimate the ratio of the relative influx of pyruvate through pyruvate carboxylase (PC) to the relative influx through pyruvate dehydrogenase (pdh) activity by the following equation:

$$pc/pdh = \frac{[G_2]+[G_3]}{[G_4]}$$

resulting in a ratio of 1.64 : 1 under our experimental conditions. This ratio is similar to that found in liver [7] and proximal tubules [14], which shows gluconeogenesis but higher than in other nongluconeogenic renal epithelial cells.

Fig. 3 also shows labeling of all carbon atoms of sorbitol and inositol. The latter results demonstrate directly that the papillary has gluconeogenic activity, as also suggested by some of the findings discussed above. Interestingly, no labeled glucose can be detected, probably because of the immediate transformation of D-glucose predominantly into sorbitol or inositol. Glycogen labeling is very low, but significant. In addition to the incubation with L[3-^{13}C]alanine we also incubated papillary tissue with [2-^{13}C]pyruvate, which was utilized 3-times better than alanine.

The ^{13}C-NMR spectrum of the supernatant (Fig. 4) of papillary tissue, which was incubated with 5 mM [2-^{13}C]pyruvate, not only indicates that inositol and sorbitol were labeled at all carbon atoms, but also glucose was completely labeled, confirming an active gluconeogenesis in papillary tissue and indicating that labeled glucose, arising from [2-^{13}C]pyruvate is the precursor of labeled sorbitol and inositol.

In order to further substantiate the finding that papillary collecting duct cells have the potential for gluconeogenesis, the activity of one of the key enzymes of this pathway, fructose-1,6-bisphosphatase was determined biochemically. For this purpose whole tissue or papillary collecting duct cells (enriched as detailed previously [8]) were homogenized by a Polytron twice, for 20 s at 4° C). Fructose-1,6-bisphosphatase activity was determined within 4 h after preparation as described by Latzko and Gibbs [9]. Protein determination was performed according to Lowry et al. [10] with bovine serum albumin as a standard.

TABLE I

FRUCTOSE-1,6-BISPHOSPHATASE (FBPase) ACTIVITY IN RENAL PAPILLA

Activity of fructose-1,6-biphosphatase was measured at 37°C. The increase of absorbance at 340 nm was recorded over 5 min. Change of absorbance was determined in the absence (basal rate) and presence of the substrate fructose-1,6-bisphosphate. The results represent mean values ± S.D. and are given in units (μmol/min) per g protein ($n = 3$).

	Rabbit papilla	Rat papilla	Papillary collecting duct cells	Rat kidney cortex
Basal rate	2.2±0.6	1.4±0.8	1.1±0.2	–
+0.6 M FBPase	7.0±1.4	3.2±1.3	2.8±0.5	279.7±12.5

The results of these experiments are shown in Table I. Both rabbit renal papilla, the tissue used in the NMR studies, and papillary collecting duct cells isolated from rat papilla show significant fructose-1,6-bisphosphatase activity. The activity of this enzyme, which is much lower than in the renal cortex, probably escaped detection in other studies [15] because the enzyme activity was determined under suboptimal conditions. For example, a small fructose-1,6-bisphosphatase activity was also found in microdissected rat renal collecting ducts by Schmidt and Guder [16].

In conclusion, these studies indicate that rabbit kidney papillary tissue synthesizes nonperturbing organic osmolytes both from glucose and from gluconeogenic substrates such as alanine. The relative importance of these two pathways for the regulation of the intracellular concentration of the osmolytes in vivo remains to be established.

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