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PROPERTIES OF SCYLLITOL TRANSPORT IN RAT KIDNEY SLICES

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SUMMARY

The transport of scyllitol in rat kidney slices was examined. Scyllitol was actively transported as was myoinositol but (+)-inositol was not. Scyllitol uptake was competitively inhibited by myoinositol and apparent influx K_m of scyllitol and K_i by myoinositol were $1.7 \cdot 10^{-5}$ and $8.4 \cdot 10^{-5}$ M, respectively. Scyllitol uptake was also inhibited by inosose-2, dinitrophenol, ouabain and phlorizin. No effect was found with D-glucose, D-galactose, mannitol and (+)-inositol. These results suggest that scyllitol shares the transport system with myoinositol, and that the transport system has stereospecificity with regard to OH orientation.

INTRODUCTION

Scyllitol is one of the cyclitols present in mammalian tissues, [1] and occurs widely in nature. A concentration of scyllitol of up to 3 mM is maintained in kidney cortex, while the concentration in blood is 0.02 mM.

Myoinositol as a main component of cyclitols in mammals, has a role as precursor of phosphatide [2]. However, scyllitol cannot be incorporated into phosphatides [3]. This fact suggests that the OH orientation at the C-2 position is essential for cyclitols to be recognized by the phosphatide-synthesizing enzyme.

Hauser [4–7] demonstrated that myoinositol was actively transported in rat kidney slices and the uptake was Na^+- and energy-dependent and inhibited by phlorizin and dinitrophenol. However, the scyllitol transport system has not yet been studied. Therefore, we have examined scyllitol transport and found that kidney slices are able to transport scyllitol actively, as is the case for myoinositol.

MATERIALS AND METHODS

Scyllitol and inosose-2 were supplied by Mitsui Pharmaceutical Ltd. [^{3}H]-Myoinositol (35 600 dpm/nmole), [^{3}H]scyllitol(7960 dpm/nmole) and (+)- [^{3}H] inositol (1056 dpm/nmole) were generous gifts from Dr T. Komai, National Institute of Health, Tokyo. (+)-Inositol was prepared from a hydrolyzate of Kasugamycin.

The assay was carried out according to Hauser's methods [5] with modifications: Rat kidney slices (100–150 mg) were preincubated at 37 °C for 15 min in 3 ml of Krebs–Ringer–phosphate buffer which was gassed with 100 % O_2. Slices were withdrawn from the medium, blotted and transferred into 3 ml of the fresh buffer, and then incubated with labeled cyclitol for the indicated time. After the incubation, slices were quickly rinsed with ice-cold buffer and blotted. The slices were homogenized in 2 ml of 0.19 M $ZnSO_4$ followed by 1 ml of 0.5 M $Ba(OH)_2$, with a Potter homogenizer. After centrifugation, aliquots for analysis were taken from the deproteinized supernatant solution.

The accumulation of cyclitol is expressed as a distribution ratio, the ratio of nmoles/ml intracellular fluid to nmoles/ml medium. Extracellular space of tissue was measured according to the method of Rea and Segal [8]. The extracellular space and total tissue water were 25 and 80 %, respectively, of wet tissue weight.

RESULTS

As shown in Fig. 1, kidney slices incorporated scyllitol and myoinositol against a concentration in medium. During incubation for 13 min, the level reached the same as that of the medium. After incubation for 40 min, the level achieved was about three times higher in concentration than that of the medium. However, the level of (+)-inositol did not show this increase with incubation and reached only 40 % of the concentration of the medium. These results showed clearly that scyllitol as well as myoinositol was incorporated by means of an active transport system but (+)-inositol was not.

The uptake of [^{3}H] scyllitol was inhibited by up to 80 % on addition of myoinositol. Therefore, the effect of myoinositol on the kinetics of [^{3}H]scyllitol

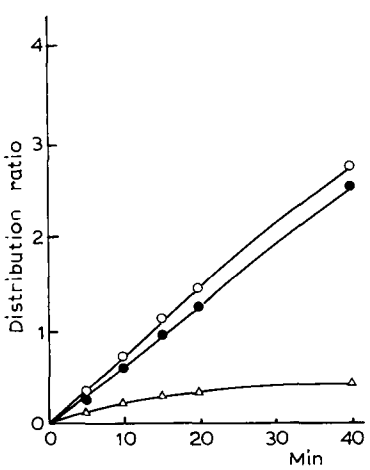

Fig. 1. Transport of myoinositol (●), scyllitol (○) and (+)-inositol (△). Rat kidney slices were incubated at 37 °C for the indicated time in 3 ml of Krebs–Ringer–phosphate buffer containing 8.4 μM of [^{3}H]myoinositol, 16.8 μM of [^{3}H]scyllitol or 42 μM of (+)-[^{3}H]inositol. Distribution ratio was determined as described in Materials and Methods.

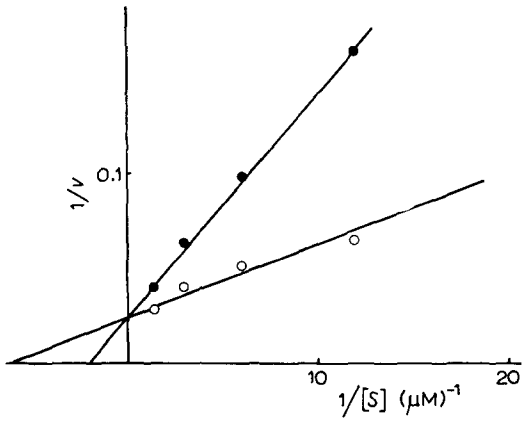

Fig. 2. Lineweaver–Burk plot of the effect of myoinositol on [^{3}H]scyllitol transport. Rat kidney slices were incubated at 37 °C for 10 min in 3 ml of Krebs–Ringer–phosphate buffer containing various concentrations of [^{3}H]scyllitol in the absence (○) or presence (●) of unlabeled myoinositol (167.5 μM). v is velocity of [^{3}H]scyllitol uptake corrected for passive transport and expressed as nmoles/ml per 10 min.

TABLE I

EFFECT OF VARIOUS COMPOUNDS ON SCYLLITOL OR MYOINOSITOL UPTAKE BY RAT KIDNEY SLICES

Rat kidney slices were incubated with 16.8 μM of [^{3}H]scyllitol or 8.4 μM of [^{3}H]myoinositol in a total volume of 3 ml of Krebs–Ringer–phosphate buffer containing the indicated material at 37 °C for 40 min. The values given are averages of 3 experiments.

Conditions	Inhibition of scyllitol uptake (%)	Inhibition of myoinositol uptake (%)
Control	0	0
Myoinositol (1.68 mM)	68	71
Scyllitol (1.68 mM)	68	75
Inosose-2 (1.68 mM)	66	63
(+)-Inositol (1.68 mM)	0	5
D-Glucose (1.68 mM)	2	4
D-Galactose (1.68 mM)	10	2
Mannitol (1.68 mM)	5	0
Ouabain (42.5 μM)	26	28
Ouabain (128 μM)	47	44
Phlorizin (64 μM)	28	25
Dinitrophenol (162 μM)	37	47

transport was investigated (Fig. 2). The apparent influx K_m was $1.7 \cdot 10^{-5}$ M. Myoinositol inhibited competitively the uptake of [^{3}H]scyllitol and the K_i value was found to be $8.4 \cdot 10^{-5}$ M.

As shown in Table I, the uptake of scyllitol or myoinositol was inhibited with dinitrophenol, an inhibitor of energy-linked uphill transport, and ouabain, an

inhibitor of sodium pump. Phlorizin inhibited the transport to a lesser extent than glucose transport. Inosose-2 was also an effective inhibitor of the transport. However, D-glucose, D-galactose, mannitol and (+)-inositol had no effect on the uptake of scyllitol and myoinositol.

To determine whether the incorporated scyllitol was present in the tissue as a non-metabolized form, an effort was made to detect radioactive compounds in the incubated slices. Radioactivity was not detected in chloroform–methanol (2:1, v/v)-soluble fraction. The water-soluble fraction was subjected to paper chromatography (pyridine-*n*-butanol–water; 1:1:1, by vol.). The radioactivity was detected mostly in one spot, the R_F value of which was identical with that of scyllitol, and radioactivity was not detected at positions corresponding to D-glucose and D-glucuronic acid. Moreover, volatile radioactivity was not detected. These results showed that scyllitol was not metabolized within the time in which the experiments were carried out.

DISCUSSION

In mammals, the studies of sugar transport have been carried out using muscle, red cells, fat cells, intestine and kidney. In the case of kidney, reabsorption of sugars as glucose and galactose from proximal renal tubules has physiological importance. Recently, in vitro investigation of sugar transport by renal cortical cells has been reported in detail [9–14]. These results showed that sugar transport was phlorizin-sensitive and needed Na^+, and that D-glucose and D-galactose were incorporated by the same carrier system.

α-Methyl-D-glucose and 5-thioglucose, which are not metabolized, have been used for studies on the kinetics and mechanism of sugar transport [15,16]. McNamara and Segal [17] noticed that the transported D-galactose was metabolized so rapidly by renal cortical cells that an analysis of the transport kinetics could not be made. The experiment reported here was carried out under conditions where scyllitol metabolism was negligible.

Johnstone and Sung [18] reported that myoinositol transport in Ehrlich ascites tumor cells was dependent on Na^+ and K^+ and was inhibited by uncoupling agents such as dinitrophenol, and that D-glucose was essentially in the accumulation of myoinositol in cells under anaerobic conditions. Caspary and Crane [19] investigated the effect of D-glucose on the uptake of myoinositol with hamster intestine. The uptake was inhibited by D-glucose but the inhibition was not of competitive type. In our experiments, the transport of scyllitol was inhibited significantly by myoinositol and inosose-2 but not by D-glucose, D-galactose, mannitol or (+)-inositol. This fact shows that the carrier of scyllitol is different from that of glucose or galactose and that the carrier is not able to recognize OH orientation at the C-2 position of cyclitol. However, the carrier is able to recognize OH orientation at C-4 and C-5 positions. Moreover, scyllitol uptake was competitively inhibited by myoinositol. This fact suggests that scyllitol and myoinositol are transported by the same carrier.

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