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PARTICIPATION OF THE RING OXYGEN IN SUGAR INTERACTION WITH TRANSPORTERS AT RENAL TUBULAR SURFACES

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Summary

The pulse-injection indicator-dilution technique in vivo has been used to study the interaction of 5-thio-D-glucose and methyl-β-D-thiogalactopyranoside with renal tubular surfaces in dog kidney.

(i) 5-Thio-D-glucose and methyl-β-D-thiogalactopyranoside have no antiluminal interaction.

(ii) 37 ± 5% of 5-thio-D-glucose is extracted at the luminal surface relative to simultaneously filtered creatinine.

(iii) Luminal extraction of 5-thio-D-glucose is blocked by preloading with D-glucose and phlorizin.

(iv) Methyl-β-D-thiogalactopyranoside in contrast to D-galactose has no luminal interaction.

It is concluded that 5-thio-D-glucose shares the glucose transporter at the luminal surface of the proximal tubule. The data also suggest that the ring oxygen participates in the interaction of pyranosides with luminal and antiluminal membrane carriers. At the luminal surface, its absence is quantitatively important while at the antiluminal surface it is apparently essential for the sugar-transporter interaction.

Introduction

In previous studies from our laboratory myoinositol was used as a model substrate in order to deduce the relative importance of the ring oxygen in sugar-transporter interactions at renal tubular surfaces [1]. These in vivo results indicated that myoinositol is taken up at both luminal and antiluminal surfaces in dog kidney. At the luminal membrane myoinositol has two pathways: one inhibited by D-glucose as well as low concentrations of phlorizin (micromolar range), and a second non-phlorizin-sensitive extraction process. Antiluminal

uptake of myoinositol is blocked by phlorizin in the millimolar concentration range [2] and may share a common transport mechanism with D-glucose and other sugars at the basolateral surface.

The present report deals with the use of the radioactively labelled 5-thio-D-[^{3}H]glucose and [^{14}C]methyl-β-D-thiogalactopyranoside to reassess the relative importance of the ring oxygen in pyranoside interaction with sugar transport receptors.

Our results indicate that 5-thio-D-glucose is extracted at the luminal surface of the nephron. This luminal extraction is blocked by D-glucose and phlorizin. However, at plasma glucose concentrations of approx. 5 mM the fractional extraction of tracer 5-thio-D-glucose (relative to a glomerular marker) is only about 35% compared to greater than 95% for D-glucose [3]. These data confirm the importance of the ring oxygen as participant in sugar-substrate interaction with the glucose transporter at the brush border membrane of the proximal tubular cell [1]. Moreover 5-thio-D-glucose does not interact significantly with the antiluminal nephron surface. Thus, the oxygen in the pyranose ring must be an essential requirement for D-glucose entry across the antiluminal membrane. This is a new finding and suggests that the previously observed antiluminal uptake pathway for myoinositol must occur via a separate process, distinct from the sugar carrier mechanism [3] at the basolateral surface.

Methyl-β-D-thiogalactopyranoside, when compared to D-galactose has neither a significant luminal nor antiluminal interaction. This result is consistent with the previously documented specificity characteristics for sugar interaction with luminal and contraluminal carriers [4] only if it is postulated that the sulphur substituent at the C-1 position alters the reactivity of the adjacent ring oxygen or some other key functional hydroxy group thereby affecting their participation in the substrate-transporter interaction (and/or transport) process.

Methods and Materials

Surgical procedure and experimental design of in vivo studies

The experimental method is the pulse-injection multiple indicator-dilution technique. For the present study, fasting mongrel dogs of either sex are anesthetized with pentobarbitol 30 mg/kg (Abbot, Montreal, Quebec) and maintained with intermittent injections (30 mg intravenously every 30 min) of this drug. The animals are intubated and artificially ventilated with periodic hyperventilation to prevent atelectasis. Muscle paralysis is maintained with anectine (Burroughs Wellcome, LaSalle, Quebec). A femoral vein catheter is used for administration of intravenous fluids (see below) and medications. Sampling catheters are placed in the left renal vein, and both right and left ureters via a midline abdominal incision as previously described [3] after which the abdomen is closed with double-layer sutures. The origin of the left renal artery at the aorta is exposed via a flank incision to provide access for the closed intra-arterial injections. Blood pressure is monitored continuously by a mercury manometer via a femoral arterial line.

Small amounts of 0.9% NaCl are administered to replace blood and fluid losses. Additional volumes of 10% mannitol are given in order to initiate a brisk diuresis. Heparinized saline (4 units/ml of 0.9% NaCl) is infused slowly to main-

tain the patency of the renal vein catheter between experimental runs.

An experimental run consists of making a pulse injection of (0.3—0.4 ml) a test solution (see below) into the left renal artery (as previously described) and obtaining timed serial samples simultaneously from the left renal vein (via a Sigmamotor pump set at 1 ml/s) and from both right and left ureters. The usual collections consist of 30 blood samples at a rate of 0.6 s/sample and 30 urine samples from each ureter at approx. 10 s/sample. The renal venous collection vials have been precoated with heparin to prevent clotting. The urine collection vials contain ampicillin to prevent bacterial growth. Arterial whole blood and plasma glucose determinations are carried out in duplicate samples drawn at the time of each experimental run. At the end of each experiment the left kidney is removed, weighed, and examined for any gross abnormalities.

Injection solution composition

The injection solution is composed of 36 mg NaCl, 100 mg bovine serum albumin (Sigma, St. Louis, MO) and 60 mg creatinine (Pfanstiehl, Waukegan, IL) in 4 ml of distilled deionized water. To this is added approx. 100 μCi of ^{3}H-labelled sugars and approx. 10 μCi of ^{14}C-labelled sugar (5-thio-D-[^{3}H]glucose and [^{14}C]methyl-β-D-thiogalactopyranoside as one combination, or [^{14}C]-methyl-β-D-thiogalactopyranoside and D-[^{3}H]-galactose as another). 1 ml is removed and will henceforth be referred to as the 'urine injection solution'. To the remaining 3 ml are added 7.5 mg Evans Blue Dye (T1824) (Matheson Coleman and Bell, Norwood, OH). This portion will be called the 'blood injection solution'.

Analytical procedures

The concentration of each of the injected indicators (T1824-albumin, creatinine, ^{14}C- or ^{3}H-labelled sugar) is measured in every blood and urine sample. The T1824-albumin serves as a plasma reference and is used to calculate plasma flow. Creatinine serves as an extracellular reference [3,5]. Using appropriate internal standards, i.e., dilutions of the blood and urine injection solutions made up in 'blank' blood and urine collected prior to each experimental run, it is possible to calculate the recovery of each of the injected indicators in all samples and to express the data as a fraction of the amount injected. For T1824-determinations, 100-μl of whole blood are diluted with 1.4 ml of 0.9% NaCl and processed as previously described [3,5]. Creatinine determinations are made on a Technicon autoanalyzer, modified to handle sample volumes of approx. 250 μl of either whole blood or urine. The reproducibility of the creatinine determinations is continuously monitored by repeating known standards at five to ten sample intervals. Thus, changes in sensitivity can be detected and appropriate corrections made. In this fashion reproducibility of each determination is better than 3%. Blood radioactivity measurements are made by taking 100-μl of whole blood and deproteinizing with 0.9 ml 95% ethanol; 200-μl of the alcohol filtrate are added to 10 ml Aquasol. All samples are counted in a Mark II liquid scintillation spectrophotometer. When dual isotopes are injected (^{3}H and ^{14}C) correction is made for crossover. Because all internal standards and unknowns are handled in the same way, there are only negligible differences in quenching between samples.

Urine radioactivity measurements are performed by pipetting 100 μl of urine directly into 10 ml of Bray's solution (J.T. Baker, Phillipsburg, NJ). Each urine collection vial is weighed before and after the experimental run. The product of weight times concentration yields the total fractional excretion per sample.

Plasma glucose determinations are carried out using a glucose determination kit (Sigma, Kit No. 510).

Data analysis

Methods for calculating recoveries, plasma flow and mean transit times have all been previously published [6]. Recirculation corrections for the renal vein outflow data are made by extrapolation of the first linear downslope obtained on semilogarithmic plots of fractional recovery/ml vs. time. The urine data are corrected for recirculation by subtracting right from left outflow curves. Catheter mean transit time is obtained by dividing the catheter volume (in ml) by the pumping rate in ml/s.

A limiting constraint on the 'resolution' of individual transit curves is that the analytical procedures impose a certain minimum volume per sample necessary for the appropriate measurements. Given this limitation the blood pump rate and speed of both renal vein and urine collection racks have been adjusted to provide the maximum possible number of experimental samples. Each determination is carried out only once.

By summing cumulative experimental errors, e.g., pipetting and counting statistics, it can be estimated that each experimental point is reproducible to within 3—5%. Evidence for this high reproducibility can be obtained by comparing the outflow pattern for two indicators with expected identical intrarenal distributions. We have previously documented that the transit patterns under such conditions will superimpose one on the other with good precision. Multiple examples of this situation have been presented in preceeding publications [2,3,5]. In the case of the present study yet another example is provided by the data shown in left hand panel of Fig. 1. Here the renal vein outflow curves for 5-thio-D-glucose and methyl-β-D-thiogalactopyranoside and creatinine superimpose within the 3—5% reproducibility constraints of each point.

In previous publications [3,5,7] we have also discussed criteria for distinguishing luminal from antiluminal events using the indicator-dilution technique. In the absence of significant binding to red cells or plasma proteins, a smaller renal vein recovery of test substrate compared to an appropriate extracellular reference such as creatinine in the initial outflow samples must reflect extraction (binding or transport) in the post-glomerular circulation. Since 5-thio-D-glucose, methyl-β-D-thiogalactopyranoside and creatinine diffuse free (unbound) in the extracellular space, the post-glomerular extraction process must occur at the level of the antiluminal membrane surface of the nephron. Under the same conditions, smaller urine recoveries of test substrate compared to a simultaneously filtered glomerular marker implies a luminal extraction. Furthermore, if the urine mean transit times of test sugars and creatinine are identical, then the extraction process is unidirectional. Superposition of test substrate on the extracellular marker in the renal vein and/or urine effluent, is our experimental criterion that, respectively, no antiluminal or luminal interaction has taken place during a single pass through the kidney.

Materials

5-Thio-D-[^{3}H]glucose (spec. act. 0.53 Ci/mmol), [^{14}C]methyl-β-D-thiogalactpyranoside (spec. act. 43.9 mCi/mmol), and D-[^{3}H]galactose (spec. act. 14.2 Ci/mmol) were obtained radiochemically pure from New England Nuclear. All other chemicals used were of the highest grade available.

Results

Table I summarizes the renal vein and urine outflow parameters derived from several experimental animals under control conditions in the fasted state where the ambient plasma glucose concentration is approx. 90—100 mg/dl. Typical renal vein and urine outflow patterns for 5-thio-D-glucose and methyl-β-D-thiogalactopyranoside are shown in Fig. 1. Inspection of the right-hand panel of Fig. 1 reveals that neither of these test sugars interact with the antiluminal surface since their renal vein outflow patterns superimpose point by point on the extracellular reference marker, creatinine. Averaging all of the experimental runs from Table I we find that $\bar{t}_{^3H}/\bar{t}_{CR} = 1.00 \pm 0.03$; $\bar{t}_{^{14}C}/\bar{t}_{CR} = 0.99 \pm 0.01$; $\Sigma^3H/\Sigma CR = 0.95 \pm 0.10$; and $\Sigma^{14}C/\Sigma CR = 1.03 \pm 0.04$.

The urine outflow curves (left-hand panel) which reflect luminal interactions indicate that 5-thio-D-glucose is extracted 37 ± 5% relative to that of simultaneously filtered creatinine but methyl-β-D-thiogalactopyranoside is negligibly reabsorbed under identical conditions (6 ± 7%).

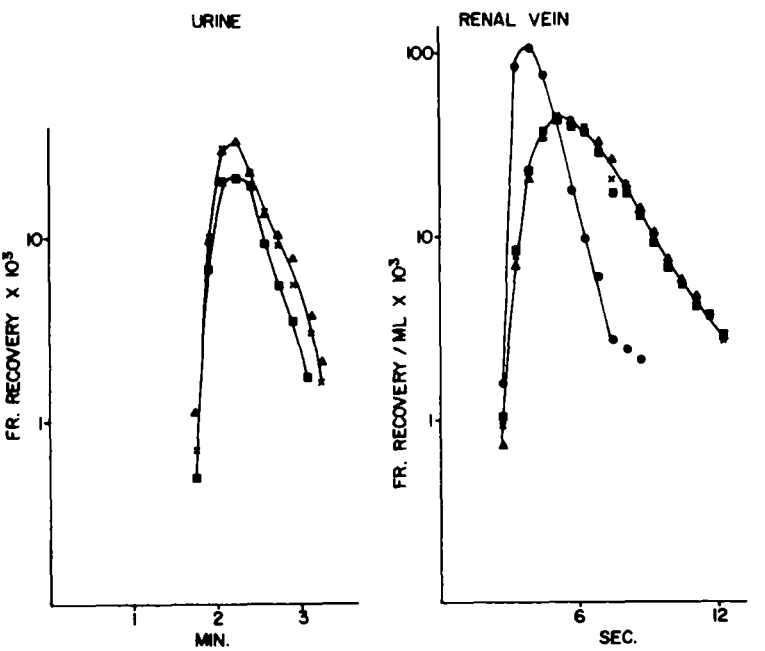

Fig. 1. Left-hand panel: simultaneous urine transit curves for creatinine (▲), 5-thio-D-[^{3}H]glucopyranoside (■), and [^{14}C]methyl-β-D-thiogalactopyranoside (X). Right-hand panel: simultaneous renal vein outflow curves for T1824-albumin (●), creatinine (▲), 5-thio-D-[^{3}H]glucose (■) and [^{14}C]methyl-β-D-thiogalactopyranoside (X).

TABLE I

Date	Run	Dog wt. (kg)	Hema-tocrit (%)	Plasma glucose (mg/dl)	Plasma flow (ml/s)	Renal vein							Urine				
						Mean transit times *				Recovery **			Recovery			Fractional reabsorption ***	
						$\bar{t}_{T1824}$	$\bar{t}_{CR}$	$\bar{t}^{3}H$	$\bar{t}^{14}C$	ΣCR	$\Sigma^{3}H$	$\Sigma^{14}C$	ΣCR	$\Sigma^{3}H$	$\Sigma^{14}C$	^{3}H	^{14}C
13/2/78	1	16	32	98	2.48	—	—	—	—	—	—	—	0.196	0.140	0.196	0.28	0
	2	16	31	91	2.72	3.61	6.66	6.54	6.54	0.747	0.693	0.779	0.265	0.161	0.218	0.39	0.18
27/2/78	2	12	39	83	4.54	2.75	5.13	4.99	5.02	0.873	0.834	0.881	0.137	0.083	0.130	0.39	0.05
13/3/79	1	14	29	90	—	—	—	—	—	—	—	—	0.149	0.085	0.136	0.43	0.09
05/3/79	1	19.5	36	—	3.75	3.78	7.36	7.33	7.29	0.769	0.686	0.794	—	—	—	—	—
	2	11	36	87	3.60	4.17	7.40	7.43	7.44	0.770	0.652	0.754	0.240	0.159	0.234	0.34	0.02
19/3/79	1	13	31	110	3.35	4.09	7.17	7.47	7.03	0.901	0.993	0.981	0.201	0.129	0.200	0.36	0.01

* Mean transit times are corrected for the catheter mean transit times.

** Recoveries are calculated as fractions of the T1824 recovery which is assumed to be totally recovered.

*** Fractional reabsorption is calculated from unity minus the fractional recovery of ^{3}H or ^{14}C relative to simultaneously filtered creatinine.

TABLE II

EFFECT OF GLUCOSE AND PHLORIZIN LOADING

Fractional reabsorption of 5-thio-D-glucose (TDG) was calculated as indicated in the legend to Table I.

Date	Run	Dog. wt. (kg)	Hematocrit (%)	Plasma glucose (mg/dl)	Fractional reabsorption of TDG	Phlorizin infusion rate (μg/kg per min)(Xmin)
06/03/78	2	14.5	32	762	0.09	0
05/03/79	3	19.5	34	335	0.13	0
13/03/78	2	14	29	83	0.05	175 (X27 min)
19/03/79	2	13	34	88	0.03	59 (X27 min)

Table II summarizes the effects of preloading animals with D-glucose and phlorizin. Comparison with Table I indicates that the urine recovery of 5-thio-D-glucose now approaches that of simultaneously filtered creatinine. In other words, raising the plasma glucose concentration or preloading with phlorizin reduces the fractional reabsorption of 5-thio-D-glucose towards zero. Fig. 2 is a representative example comparing the urine outflow curves for 5-thio-D-glucose, methyl-β-D-thiogalactopyranoside and creatinine before and after systemic infusion of phlorizin. Phlorizin blocks the luminal extraction of 5-thio-D-glucose as indicated by the fact that the urine transit pattern for 5-thio-D-glucose superimposes on the glumerular reference.

Modifications at the C-1 position, i.e., α-methyl-D-glucopyranoside, have no effect on the specificity of interaction with the luminal (brush border) D-glucose

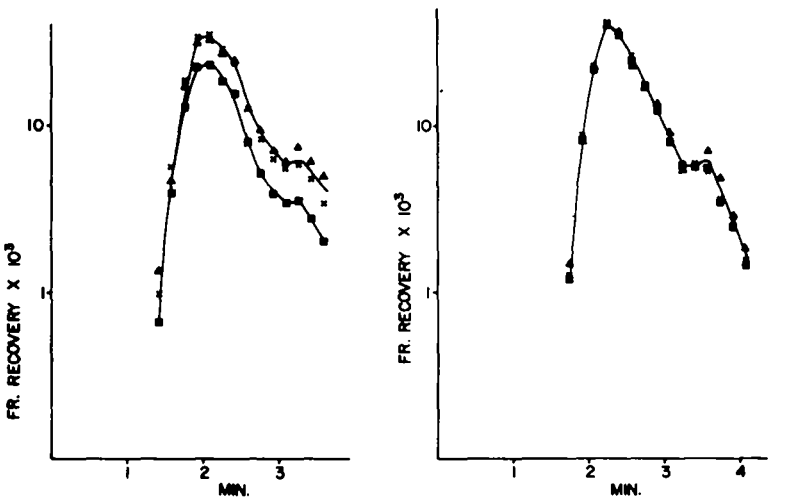

Fig. 2. The effect of phlorizin on the luminal extraction of 5-thio-D-glucopyranoside. Left-hand panel: simultaneous urine outflow curves for creatinine (▲), 5-thio-D-[^{3}H]glucose (■) and [^{14}C]methyl-β-D-thiogalactopyranoside (X) under control conditions. Plasma glucose concentration 110 mg/dl. Right-hand panel: simultaneous urine outflow curves for the same compounds under conditions of phlorizin infusion of 59 μg/kg body wt. per min for 27 min. Plasma glucose concentration 88 mg/dl.

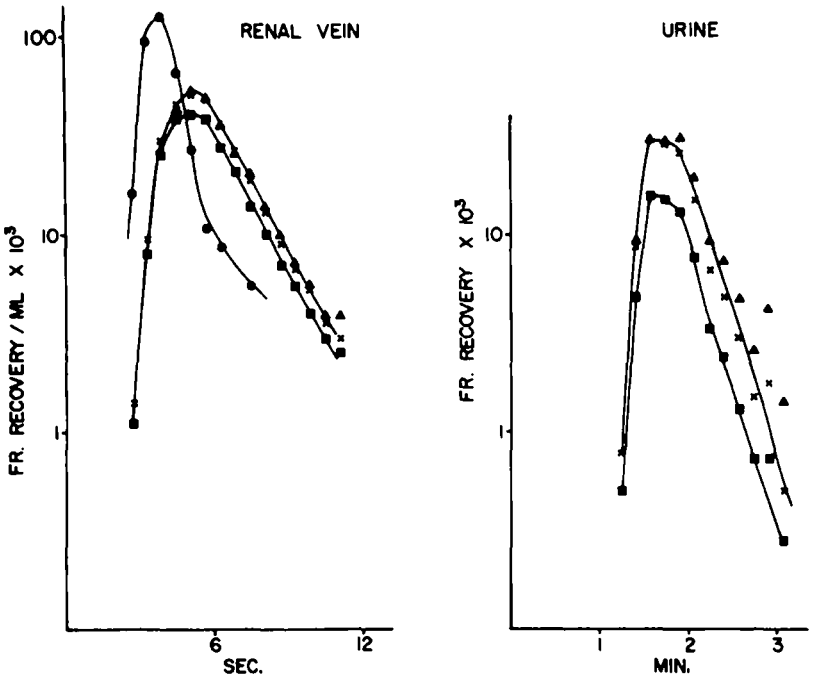

Fig. 3. Left-hand panel: simultaneous renal vein curves for T1824-albumin (•), creatinine (▲) [^{3}H]galactose (■) and [^{14}C]methyl-β-D-thiogalactopyranoside (X). Right-hand panel: urine transit curves for the same indicators.

transporter [1,4] but changes at C-1 do abolish the antiluminal membrane interaction [1,8]. Similarly, β-methyl-D-galactopyranoside is a more effective competitive inhibitor of the brush border membrane high-affinity phlorizin receptor than is D-galactose [7]. On the basis of this evidence we would have predicted that methyl-β-D-thiogalactopyranoside should exhibit the same luminal interaction as D-galactose [5] but that its antiluminal interaction would be abolished.

Fig. 3 shows the results of an experimental run in which methyl-β-D-thiogalactopyranoside and D-galactose were injected simultaneously together with T1824-albumin and creatinine. As in Fig. 2, methyl-β-D-thiogalactopyranoside superimposes on creatinine in the renal vein outflow. This implies no antiluminal interaction. But as documented previously [5], D-galactose emerges under creatinine on the upslope and peaks lower, indicating contraluminal uptake. This finding is therefore consistent with the postulation that a C-1 hydroxyl group is essential for sugar interaction with its antiluminal membrane transporter [4,5].

Inspection of the urine outflow curves in Fig. 3 shows that whereas D-galactose is extracted by about 50% at the luminal surface, methyl-β-D-thiogalactopyranoside is negligibly reabsorbed under identical conditions. Thus, taken together with the data summarized in Table I our results indicate that methyl-β-D-thiogalactopyranoside has no luminal interaction.

Discussion

In canine and human brush border membrane preparations, 100 mM thio-D-glucose inhibits high-affinity phlorizin binding and D-glucose uptake [9,10]. Also, 5-thio-D-glucose shares the D-glucose transporter in the rat intestine [11].

The present in vivo study using radioactively labelled tracer 5-thio-D-glucose provides further evidence that 5-thio-D-glucose shares the D-glucose transport mechanism at the luminal surface of the proximal tubule because the fraction extracted at the luminal surface is blocked by glucose and phlorizin loading. This result is also consistent with our previous finding that myoinositol shares the D-glucose transporter at the luminal membrane. It is apparent that the ring oxygen must participate in the interaction of pyranoses with the brush border D-glucose transporter because its replacement by a sulphur atom reduces the fractional extraction in single pass experiments from greater than 95% to less than 40%.

Since all previous evidence points to the fact that modifications at the C-1 position do not affect the handling of, for example, α- or β-methyl-D-glucoside or β-methylgalactopyranoside by the luminal glucose transporter, how can we explain the fact that methyl-β-D-thiogalatopyranoside is negligibly reabsorbed at the luminal surface relative to D-galactose? It has been amply documented that 1-thiosugars have a different chemical reactivity compared to the non-substituted sugars [12]. Specifically, the sulphur is less basic and more easily polarized than an oxygen atom at the C-1 position. This difference may be reflected in an altered dipole-dipole interaction between the C-1 sulphur substituent and the ring oxygen. Given the importance of the ring oxygen in the substrate-transporter interaction it seems reasonable to postulate that subtle changes in the charge distribution around the ring oxygen are responsible for making methyl-β-D-thiogalactopyanoside a non-interacting substrate at the brush border. Alternatively, small changes in charge distribution might occur at other locations in the pyranose ring, thereby affecting participation of important functional hydroxyl groups in the interaction with the carrier.

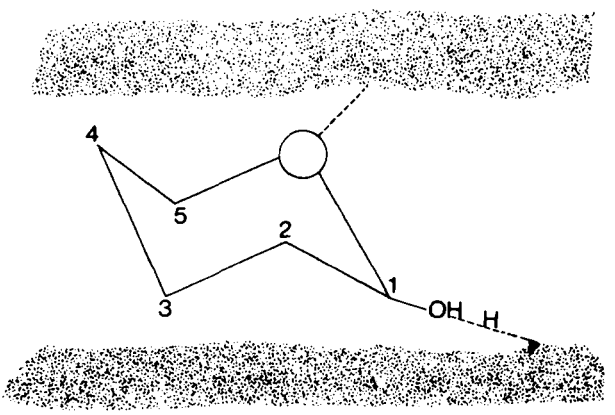

Fig. 4. Schematic diagram indicating essential points of interaction between a pyranose substrate and the glucose transporter G′ (see Fig. 5) located at the antiluminal surface of the proximal tubule.

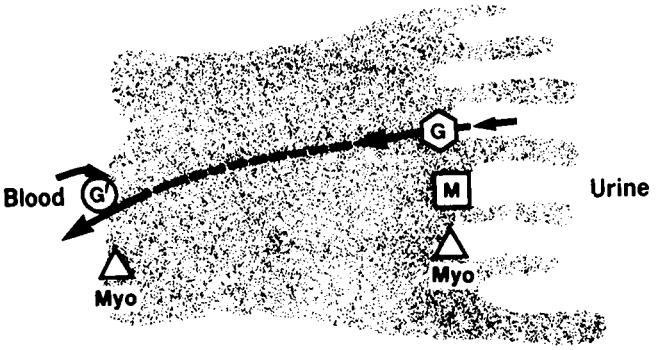

Fig. 5. Schematic diagram indicating the different sugar transport receptors postulated at both brush border and antiluminal surfaces of the renal proximal tubule in canine kidney. G is the glucose transporter (Na^+-dependent, phlorizin-sensitive) at the brush border membrane, M is the mannose transporter, Myo represents a specialized system for myoinositol. G′ represents a shared transport system for glucose and other sugars at the antiluminal surface of the nephron (Na^+-independent, phlorizin-insensitive). In addition, based on the present study, there must also exist a separate myoinositol transport pathway at the basolateral surface. The arrows indicate that under normal in vivo conditions, a flux of sugars via the G transporter across the brush border membrane is essentially unidirectional because of the transmembrane electrical potential gradient. At the basolateral membrane, the sugar transport system is equilibrative.

In previous experiments [1] we have found that the hydroxyl group at the C-1 position is an essential requirement for sugar interaction with the antiluminal membrane. It was therefore expected that methyl-β-D-thiogalactopyranoside should have no contraluminal uptake compared to D-galactose. However, we must now reconcile the absence of an interaction between 5-thio-D-glucose and the antiluminal membrane with the fact that myoinositol is taken up at the contraluminal surface by a mechanism that is inhibited by large systemic doses of phlorizin [2].

Since 5-thio-D-glucose more closely resembles D-glucose than myoinositol, it is a better model substrate to test the extent of the participation of the ring oxygen in the interaction with the sugar transporter mechanism. We must therefore revise our previously assigned antiluminal membrane specificity characteristics for sugar transport. It now seems clear that the ring oxygen and the C-1 hydroxyl group are both essential participants in the interaction of sugars with contraluminal carrier (see Fig. 4). This means that except for a relatively minor luminal pathway, shared with D-glucose, tubular uptake of myoinositol across both brush border and antiluminal surfaces occurs via mechanisms which are independent of the glucose carrier (see Fig. 5). The existence of these specialized transporter systems emphasizes the importance of the kidney as a metabolic sink for myoinositol, and explains why in end-stage renal disease plasma myoinositol levels are very high [13]. The existence of at least three different pathways for myoinositol entry into the renal tubular cell may explain the detailed kinetics observed in a vesicle membrane preparation from rat kidney [14].

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