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THE CHEMICAL AND STERIC DETERMINANTS GOVERNING SUGAR INTERACTIONS WITH RENAL TUBULAR MEMBRANES*

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SUMMARY

1. The multiple indicator dilution technique in vivo has been used to study the specificity of interaction of pyranoside derivatives with renal tubular surfaces in dog kidney. The substances used include [^{14}C]-methyl-α-D-glucoside, [^{3}H] methyl-β-D-glucoside, D-[^{14}C]mannosamine, D-[^{14}C]fructose, L-[6-^{14}C]idose, D-[^{3}H]-allose, [2-^{3}H]*myo*-inositol, [^{14}C]glucosamine–HCl and D-[6-^{3}H]mannosamine hydrochloride.

2. For the luminal (brush border) membrane the specificity characteristics consist of: (i) a pyranose ring in a chair conformation (ii) OH groups on carbons 3 and 6 oriented as in configuration of D-glucose.

3. For the antiluminal membrane the specificity characteristics are (i) a pyranose ring in a chair conformation (ii) hydroxyl groups on carbons 1 and 2 (iii) OH groups if present on carbons 3 and 6 should be oriented equatorially as in the configuration of D-glucose.

4. These data are compatible with the specificity of inhibition of high affinity phlorizin receptors in vitro, and suggest that these receptors are identical to the sites associated with the glucose-transport mechanism.

5. The results also suggest that the dominant feature controlling the dynamics of the interaction between sugars and membrane-bound transport receptors is the position of certain functional hydroxyl groups relative to the plane of the pyranose chair (C1 or 1C), defined by carbons 2, 3, 5, and the ring oxygen. In this regard the axial orientation of a single OH group on the pyranoside may be of special importance to the sugar–receptor interaction.

INTRODUCTION

Previous studies have demonstrated the existence of at least two transport systems for sugars in the proximal tubule: one located at the luminal membrane (brush border) and one at the antiluminal (peritubular) surface [1,2].

Competitive inhibition experiments [2] have shown that the sugar transport system at the brush border contains two sets of receptor sites. One site has a high

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affinity for phlorizin, is shared by D-glucose, 2-deoxy-D-glucose, and D-galactose, and is referred to as the glucose-transport receptor (G receptor). The second set of receptors has D-mannose as the preferred substrate (M receptor).

Recently, several laboratories have described a high affinity binding receptor for ^{3}H-labelled phlorizin in an enriched brush border membrane fraction prepared from rat kidney [3–5]. The binding of phlorizin in vitro exhibits remarkable similarity to the specificity of the G-transport receptor in dog kidney [2,4,5]. Thus it appears that the membrane-bound high-affinity phlorizin receptors are identical to sites associated with the true glucose transporters.

This same conclusion has been reached by Vick et al. [6]. Using the micropuncture technique in the rat, these authors studied the inhibition of active glucose transport by phlorizin analogs. They then compared the inhibition constants (K_i) for these compounds in vivo with the results of a similar study on the inhibition of [^{3}H]phlorizin binding to isolated brush border fragments [5]. There was a remarkably good quantitative and qualitative correlation between their in vivo and in vitro studies.

The purpose of the present paper is to extend our in vivo dog studies employing radiolabelled pyranose derivatives to probe the specificity of interaction of monosaccharides with renal tubular surfaces (antiluminal and luminal). This will enable us to apply strict specificity criteria to the binding of phlorizin to intact membranes [7] and their solubilized products [8].

The results of the present study largely substantiate the chemical and steric requirements which we have previously published [2]. However one important new feature is the finding that the Cl chair conformation may not be essential to sugar interaction with the G receptor at the brush border. The cumulative data now indicate that the chemical and steric characteristics of this interaction are more general, and consist of: (1) a pyranose ring in a chair conformation (Cl or 1C) and (2) OH groups on carbons 3 and 6 oriented as in the configuration of D-glucose.

MATERIALS AND METHODS

All studies have been carried out in vivo using fasting, anaesthetized mongrel dogs under mannitol diuresis. The basic experimental technique is the multiple indicator dilution method as previously described [1,9]. Recirculation corrections and details for calculating blood flow, mean transit times, and recoveries have been presented in previous publications [10].

All radioactive materials used in these studies were obtained commercially. The following is a list of the test radiolabelled sugars and their respective specific activities as well as source of purchase.

[^{14}C]Methyl-α-D-glucoside, 50 mCi/mmole, (Calatonic, Los Angeles, Calif.); [^{3}H]Methyl-β-D-glucoside, 200 mCi/mmole, (New England Nuclear Corp.); D-[^{14}C]-Mannosamine, 55 mCi/mmole, (Amersham-Searle); D-[^{14}C]-Fructose, 5.11 mCi/mmole, (New England Nuclear Corp.); L-[6-^{14}C]-Idose, 42 mCi/mmole, (Schwarz Bio Research); D-[^{3}H]-Allose, 975 mCi/mmole, (Amersham-Searle); [2-^{3}H]-*myo*-inostiol, 1–5 Ci/mmole, (Amersham-Searle); [^{14}C]-Glucosamine–HCl, 200 mCi/mmole, (Amersham-Searle); D-[6-^{3}H]-Mannosamine Hydrochloride, 2–5 Ci/mmole, (New England Nuclear Corp.).

Analytical determinations of T1824, creatinine and radioactivity were performed as described previously [1,2]. Pipettings were carried out using an automatic dilutor (ADD System Fisons Scientific Apparatus) having a reproducibility of 0.6%. Each experimental point has a reproducibility of better than 3%, and an experimental error which is better than 5%.

TABLE I

INDICATOR DILUTION DATA

Sugar	Dog wt (kg)	Drained kidney wt (g)	Plasma glucose (mg/100 ml)	HCT %	Plasma flow (ml/s)
[^{14}C]Methyl-α-D-glucoside	16	70	75	33	5.7
	19	45	82	35	5.20
[^{3}H]Methyl-β-D-glucoside	16	70	75	44	5.7
	19	54	82	35	5.20
D-[^{14}C]Mannitol	—	53	—	38	6.1
	—	53	—	38	5.8
L-[^{14}C]Idose	16	70	77	32	5.1
	19	54	81	34	6.4
D-[^{3}H]Allose	16	70	77	32	5.1
	19	54	81	34	6.4
D-[^{14}C]Mannosamine	14	49	69	35	2.8
	14	49	66	32	-
D-[^{3}H]Mannosamine	13	56	94	30	5.0
[^{14}C]Glucosamine-HCl	13	56	94	30	5.0
	16	42	-	31	6.2
	16	42	—	31	6.2
D-[^{14}C]Fructose	—	49	-	40	8.2
	12	47	73	30	3.3
	18	61	84	40	-
[^{3}H]*myo*-Inositol	—	53	107	38	6.1
	—	53	115	38	5.8
	11	53	58	34	-
	17	48	-	39	4.2
	17	55	111	36	4.7
	17	55	130	31	-

* ΣCR and $\Sigma^{14}C(^{3}H)$ represent the renal vein recoveries of creatinine and the indicated radiolabelled pyranoside relative to the recovery of T1824.

** $\bar{t}$ represents the renal vein mean transit times corrected for the mean transit time of the catheter.

*** $1 - [\Sigma^{14}C(^{3}H)/\Sigma CR]$ represents the fractional reabsorption of labelled pyranoside across the luminal membrane.

RESULTS

A summary of the data for each of the tested sugar derivatives is presented in Table I. The test sugars are compared to simultaneously injected creatinine, which is the extracellular reference marker.

Renal vein				Urine		
$\frac{\Sigma CR^{*}}{\Sigma T1824}$	$\frac{\Sigma^{14}C(^{3}H)^{*}}{\Sigma T1824}$	$\bar{t}_{CR}$ ** (s)	$\bar{t}_{^{14}C(^{3}H)}$ ** (s)	ΣCR	$\Sigma^{14}C(^{3}H)$	$1-\frac{\Sigma^{14}C(^{3}H)^{***}}{\Sigma CR}$
0.85	0.90	5.8	5.8	0.12	0.007	0.95
0.83	0.83	5.4	5.5	0.10	0.004	0.96
0.85	0.91	5.8	5.3	0.10	0.002	0.98
0.83	0.89	5.4	5.4	0.10	0.003	0.97
0.85	0.84	5.9	5.8	0.16	0.17	0.00
0.82	0.73	4.9	5.9	0.09	0.11	0.00
0.85	0.86	7.1	7.1	0.10	0.09	0.10
0.85	0.86	5.3	5.3	0.09	0.09	0.00
0.85	0.88	7.1	7.1	0.10	0.09	0.10
0.85	0.85	5.3	5.3	0.09	0.09	0.00
0.71	0.75	9.2	9.2	—	—	
0.71	0.72	7.6	7.6	0.16	0.15	0.06
0.82	0.81	6.2	6.2	0.06	0.07	0.00
0.82	0.79	6.2	6.2	0.06	0.06	0.00
0.70	0.69	6.1	6.2	0.07	0.07	0.00
0.74	0.77	6.0	5.9	0.11	0.09	0.02
0.79	0.70	6.3	6.3	0.10	0.04	0.60
0.73	0.65	6.7	6.5	0.14	0.03	0.79
—	—	—	—	0.13	0.03	0.77
0.85	0.64	5.9	5.6	0.16	0.02	0.87
0.82	0.69	4.9	5.0	0.09	0.01	0.89
—	—	—	—	0.21	0.01	0.95
0.87	0.73	6.2	6.4	0.21	0.02	0.91
0.82	0.54	5.2	5.5	0.13	0.01	0.92
—	—	—	6.2	0.13	0.01	0.92

As previously described [1,2], the experimental rationale for distinguishing luminal from antiluminal events is based upon consideration of the urine and renal vein outflow curves for the test substance relative to creatinine. Because sugar reabsorption has been localized to the proximal tubule [11] the luminal extraction of radiolabelled sugars observed in our studies must reflect events occurring at the level of the brush border membrane of the proximal tubule.

The effect of the anomeric hydroxyl group

Inspection of Fig. 1 reveals that methyl-α- and β-D-glucopyranoside are superimposable on the renal vein creatinine outflow curve. This implies that both the α and β anomers have an extracellular distribution from the postglomerular circulation (i.e. they have no interaction with the antiluminal surface of the nephron). Also, each anomeric form is extracted by the luminal membrane to the extent of approximately 95% of the amount filtered (Table I). Systemic administration of phlorizin (25 mg dissolved in 20 mM $NaHCO_3$) markedly inhibits the luminal extraction (Table II), of methyl-α- and β-D-glucopyranoside and the urine outflow patterns for these two radiolabelled sugars then tend toward coincidence with that of simultaneously filtered creatinine (Fig. 2, right-hand panel). Similarly, raising plasma glucose from 80 to 200 mg/100 ml decreases the fractional extraction of tracer methyl-α- and β-D-glucopyranoside from 95 to 60% (Table III and Fig. 2, middle panel).

Since both anomers interact with the luminal surface of the proximal tubule at a site which is also sensitive to competitive inhibition by phlorizin and glucose loading, we conclude that this site is identical to the G (glucose) transport receptor. This finding confirms our previous suggestion [2] that variations of the position of the

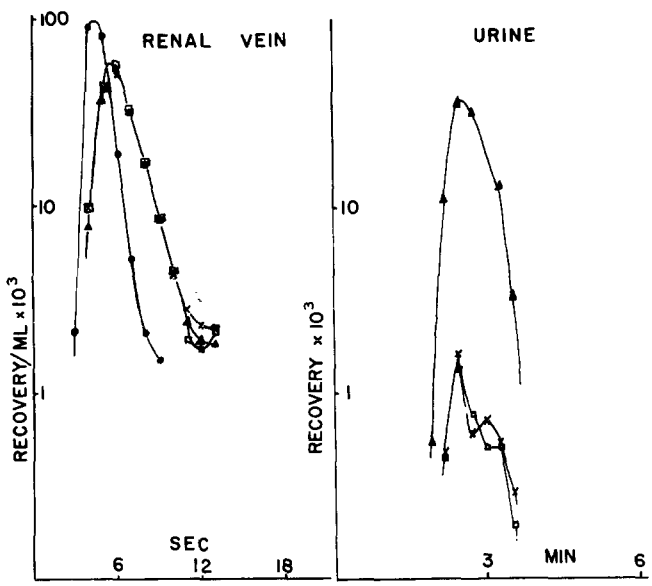

Fig. 1. Simultaneous renal vein and urine outflow curves for T1824 (●), creatinine (▲), [^{14}C]-methyl-α-D-glucoside (×), and [^{14}C]methyl-β-D-glucoside (■).

TABLE II

EFFECT OF PHLORIZIN LOADING ON MONOSACCHARIDE REABSORPTION

The results of phlorizin loading on the fractional reabsorption of tracer pyranoside is shown for systemic doses of phlorizin varying from 25 to 200 mg. Phlorizin was administered in a single bolus and the experimental run carried out after about 30 min.

	Plasma glucose (mg/100 ml)	ΣCR	$\Sigma^{14}C(^{3}H)$	$1-\frac{\Sigma^{14}C(^{3}H)}{\Sigma CR}$
[^{14}C]Methyl-α-D-glucoside	41	0.14	0.11	0.21
[^{3}H]Methyl-β-D-glucoside	41	0.21	0.16	0.23
D-[^{14}C]Fructose	42	0.21	0.16	0.23
	84	0.04	0.04	0.00
[^{3}H]*myo*-Inositol	60	0.16	0.10	0.38
	—	0.14	0.09	0.36

hydroxyl group at carbon 1 have no influence on the reactivity of monosaccharides with glucose transport receptors at the brush border.

Because the renal vein transit pattern for methyl-α- and β-D-glucopyranoside coincides with the creatinine curve, we conclude that the presence of an hydroxyl group on carbon 1 is necessary for the interaction of a pyranoside with the antiluminal membrane. Therefore we must extend our previously stated specificity

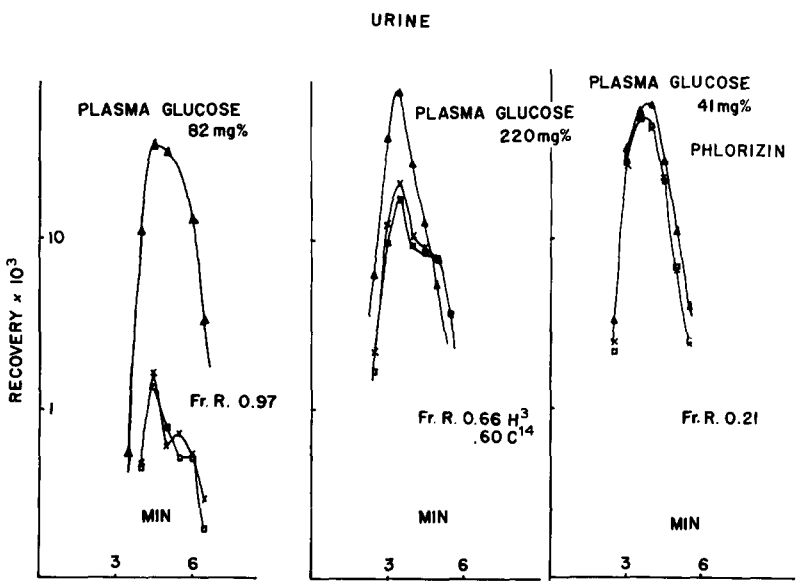

Fig. 2. Urine outflow curves for creatinine (▲), [^{14}C]methyl-α-D-glucoside (×), and [^{3}H]methyl-β-D-glucoside (■), under control conditions (left hand panel), glucose loading (middle panel), and phlorizin loading (right hand panel).

TABLE III

EFFECT OF GLUCOSE LOADING ON MONOSACCHARIDE REABSORPTION

The results of increasing the filtered load of glucose on the fractional reabsorption of tracer pyranosides are indicated by the effect of raising plasma glucose concentration (compare Table I and III).

	Plasma glucose (mg/100 ml)	ΣCR	$\Sigma^{14}C(^3H)$	$1-\frac{\Sigma^{14}C(^3H)}{\Sigma CR}$
[^{14}C]Methyl-α-D-glucoside	266	0.07	0.03	0.57
	310	0.17		0.59
[^{3}H]Methyl-β-D-glucoside	266	0.07	0.03	0.57
	310	0.17		0.65
D-[^{14}C]Fructose	203	0.15	0.11	0.27
[^{3}H]*myo*-Inositol	640	0.09	0.09	0.00
	220	0.14	0.06	0.57

characteristics governing sugar–antiluminal interactions [2] to include the requirement for a hydroxyl group on carbon 1 as well as on carbon 2.

The chair versus straight chain conformation of hexoses

D-Mannitol exists in aqueous solution in a straight chain conformation [12]. Inspection of Fig. 3 reveals that the renal vein and urine outflow transit patterns for mannitol superimpose on creatinine. Therefore D-mannitol has an extracellular

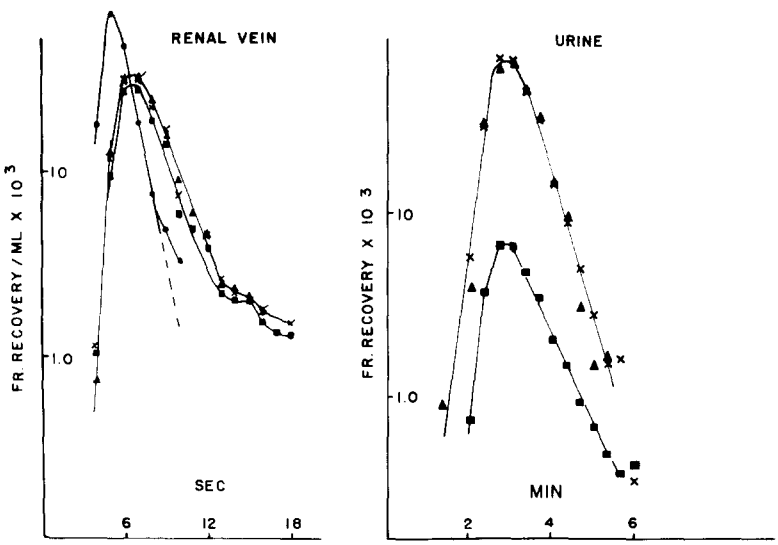

Fig. 3. Simultaneous renal vein and urine outflow curves for T1824 (●), creatinine (▲), D-[^{14}C]-mannitol (×), and [^{3}H]*myo*-inositol (■).

distribution and no observable interaction with either the luminal or antiluminal surface of the nephron. This finding emphasizes that for a monosaccharide to interact with renal tubule surfaces it must be present as a pyranoside in a chair conformation.

Importance of certain functional hydroxyl groups in the pyranose–membrane interaction

The results for both L-idose and D-allose are shown in Fig. 4. These radiolabelled monosaccharides emerge in the renal vein and urine outflows coincident with the creatinine curves. Therefore L-idose and D-allose have no interaction with either the luminal or antiluminal surface of the nephron. D-Allose exists in aqueous solution in the form of a pyranose ring in the Cl chair conformation but with the hydroxyl group on carbon 3 oriented axially to the mean plane of the chair compared to the equatorial arrangement of this group in D-glucose. Since D-allose remains extracellular this means that the spatial orientation of the hydroxyl group on carbon 3 with respect to the mean plane of the pyranose ring is crucial for the interaction of the pyranose ring with transport receptors located both at the luminal and antiluminal membranes, From ref. 13 we deduce that L-idose exists at equilibrium in aqueous solution predominantly in the Cl pyranose conformation. A significant fraction (about 30%) also exists in the furanose form. The fact that L-idose does not interact with renal tubular surfaces could be related to the axial orientation of the OH groups on carbons 2, 3, and 4 or to the presence of the furanose form. Evidence presented for D-allose and for D-fructose (see below) supports the former hypothesis.

The effect of amino substituents at the carbon 2 position

The effect of amino substituents at the carbon 2 position both in the axial

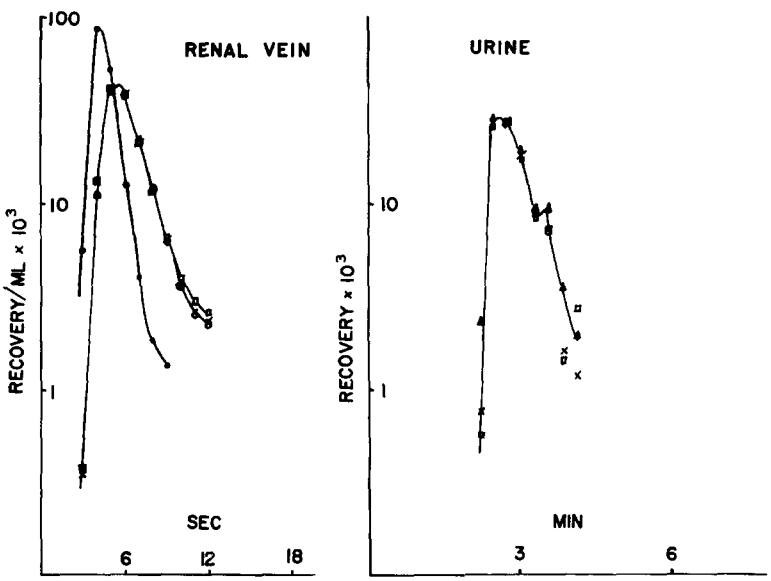

Fig. 4. Simultaneous renal vein and urine outflow curves for T1824 (●), creatinine (▲), L-[^{14}C]-idose (×), and D-[^{3}H]allose (■).

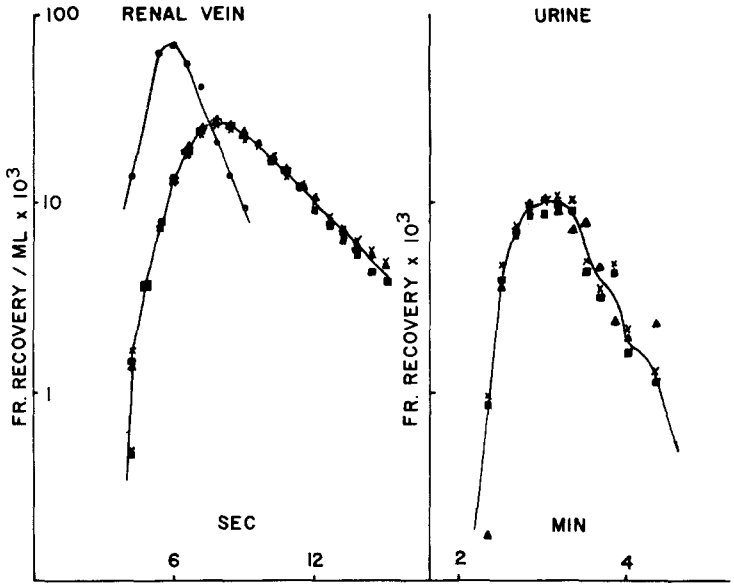

Fig. 5. Simultaneous renal vein and urine outflow curves for T1824 (●), creatinine (▲), [^{14}C]-glucosamine (×), and [^{3}H]mannosamine (■).

and equatorial position was tested by examining the renal vein and urine outflow patterns of mannosamine and glucosamine respectively. Inspection of Fig. 5 shows that these two amino sugars superimpose on the creatinine effluents in both the renal vein and urine and thus have no interaction with either the luminal or antiluminal surface of the nephron. We cannot be certain from our studies whether the lack of reactivity of these amino sugars for the glucose transport receptors is based on the bulkiness of the amino substituent group at the carbon 2 position, or because of their state of protonation. The latter would be dependent upon the pH at the site of membrane interaction. This lack of reactivity of labelled amino sugars with the glucose transport receptors in the dog kidney in vivo correlates with the in vitro findings of Barnett [14] in the hamster intestine, in which amino sugars were found not to significantly inhibit glucose transport.

The interaction of ketohexoses

Fig. 6 shows the transit curves for D-fructose relative to creatinine, and T1824. In the renal vein, D-fructose emerges so that it lies below the creatinine curve. In other words, its recovery in venous blood is less than that of creatinine and its mean transit time is shorter (see Table I). Our renal vein data for fructose is essentially the same as that found by Chinard in an earlier study [15]. This behaviour is also reminiscent of the renal vein data obtained for hippurate, a secreted substance [11]. Therefore we conclude that fructose interacts with the antiluminal surface of the nephron. Inspection of the urine outflow pattern reveals that the recovery of [^{14}C] fructose is less than simultaneously filtered creatinine. This implies an extraction by the luminal surface of the nephron. Fig. 7 illustrates the urine outflow curves for fructose under conditions of phlorizin and glucose loading. Both phlorizin and

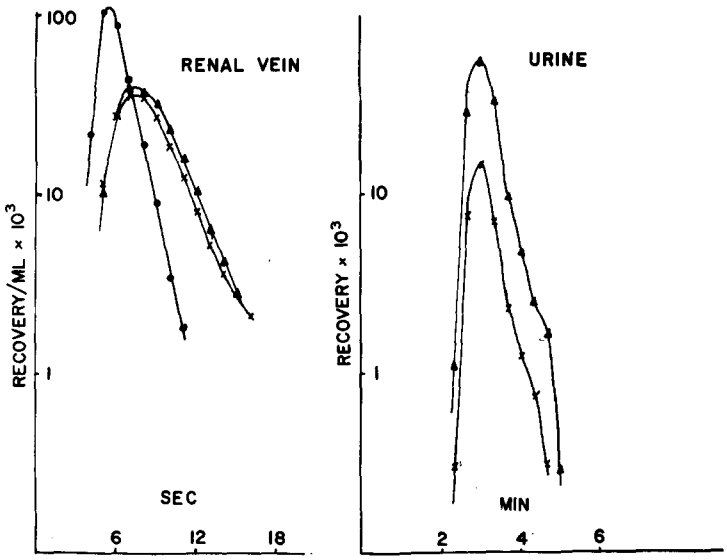

Fig. 6. Simultaneous renal vein and urine outflow curves for T1824 (●), creatinine (▲), and [^{14}C]fructose (×).

glucose markedly inhibit fructose interaction with the luminal membrane causing the urine fructose and creatinine curves to become almost superimposable (Tables II and III).

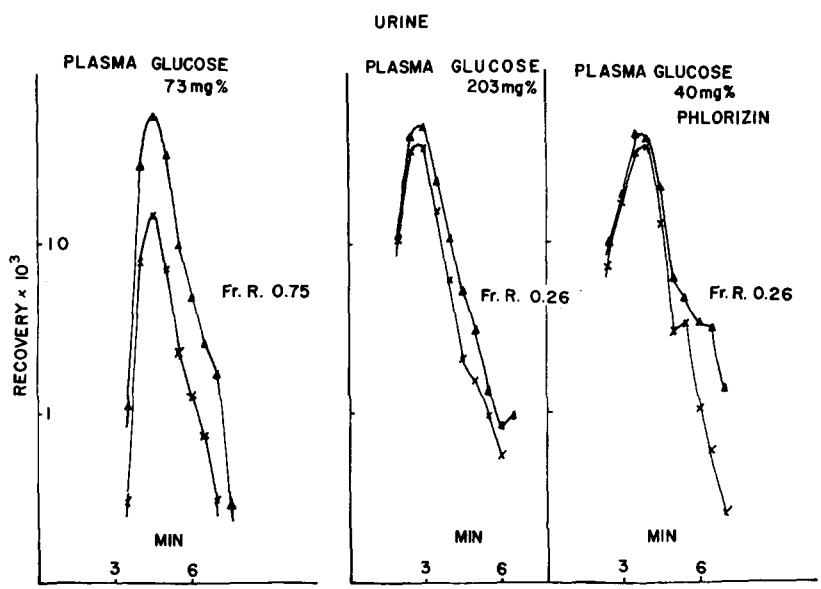

Fig. 7. Urine outflow curves for creatinine (▲), and [^{14}C]fructose (×), under control conditions (left-hand panel), glucose loading (middle panel), and phlorizin loading (right-hand panel).

Thus fructose interacts with the brush border of the proximal tubule at the same sites as D-glucose (i.e. the G receptor).

In these same studies, the low doses (50 mg) of phlorizin used and the minimal raising of plasma glucose concentration, did not affect the interaction of fructose with the antiluminal membrane. The relative insensitivity of fructose interaction with the antiluminal membrane to inhibition by phlorizin is similar to our previous experiments [1] where it was shown that the dose of phlorizin required to inhibit D-glucose interaction with the antiluminal membrane is about 1000 times that required to block glucose transport at the brush border.

The experimental data therefore indicate that fructose is taken up at both surfaces of the renal proximal tubule. The decreased total recovery of [^{14}C]fructose could be due to either a concentrative uptake within the renal tubule or first uptake followed by metabolic degradation to $^{14}CO_2$ and water.

Our conclusion that D-fructose, a keto sugar, shares the G transport receptor might seem to contradict the stated requirement that for a monosaccharide to interact with the G site it must exist at equilibrium as a pyranose ring in a chair conformation. However in aqueous solution about 60% of D-fructose exists as a pyranoside with the β anomeric form predominating (57%) [16]. Moreover the most stable form of β-D-fructose is the 1C conformation. The Corey-Pauling-Koltun (CPK) space-filling molecular model of β-D-fructose (in the 1C conformation) is shown in Fig. 8 along with D-glucose. Inspection reveals that the two molecules can be oriented so that the left hand portions of the two molecules are identical. In contrast, α-D-fructopyranoside is most stable in the C1 conformation [17]. But inspection of CPK models of the α anomeric form in the C1 conformation reveals little similarity to D-glucose. Thus if we try to correlate the common structural and steric features of fructose and glucose with the observation that D-fructose shares the glucose transport receptor at the brush border, it would seem that the keto-hexose must interact with the transport mechanism in the form of β-fructopyranoside

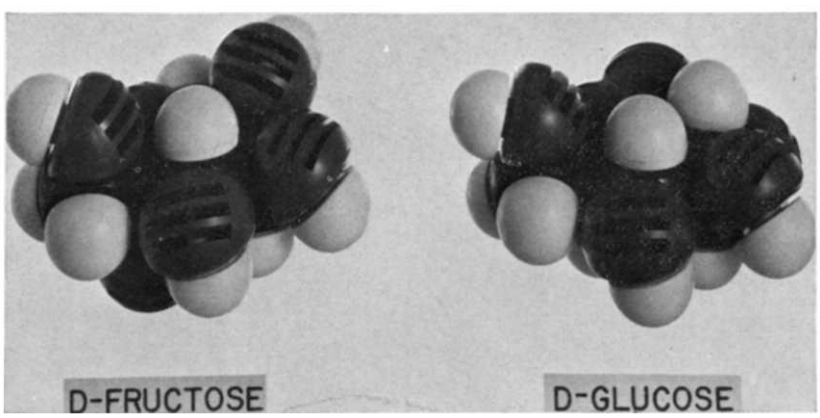

Fig. 8. CPK space-filling molecular models of D-fructose (left) and β-D-glucose (right). Fructose has been constructed in the 1C conformation in the β anomeric form. The molecular model for fructose has been oriented in space to show its structural similarities to the left-hand portion of the glucose molecule. This part of glucose contains the OH groups on carbons 6 and 3 which are essential for monosaccharide–membrane interactions at the brush border.

(1C). Based on these stereochemical similarities, the specificity requirement for OH groups on carbons 3 and 6 of D-glucose could be satisfied by the hydroxyl groups on carbons 2 and 4 of β-D-fructose. Thus the geometric relationships of the functional OH groups relative to the mean plane of the pyranose ring may be more important than the type of chair conformation (C1 or 1C) in determining sugar interaction with specific transport receptors.

The effect of the ring oxygen on transport specificity

Fig. 3 shows the renal vein and urine outflow curves for [^{3}H]*myo*-inositol. compared to creatinine and T1824. The data for *miyo*-inositol are very similar to that obtained for fructose. *myo*-Inositol emerges in the renal vein below creatinine indicating that it interacts with the antiluminal surface of the nephron. Also the urine recovery of labelled *myo*-inositol is less than that of simultaneously filtered creatinine, implying that *myo*-inositol is also extracted by the luminal surface. Fig. 9 shows the urine outflow curves obtained under control conditions and then following the systemic administration of phlorizin and glucose. Both phlorizin and glucose loading markedly decrease the fractional extraction of tracer *myo*-inositol by the luminal surface of the nephron (Tables II and III). Therefore most of the labelled *myo*-inositol must share the G receptors located at the brush border surfaces of the proximal tubule. We conclude that the substitution of a carbon atom for oxygen in the pyranose ring has no effect on the reactivity of pyranosides with G receptors. These results are consistent with the experimental findings that 5-thio-D-glucose and *myo*-inositol are both transported in the hamster intestine [18,19].

Certain other features of the behaviour of *myo*-inositol are illustrated in Fig. 9, where we show the urine outflow curves for simultaneously injected

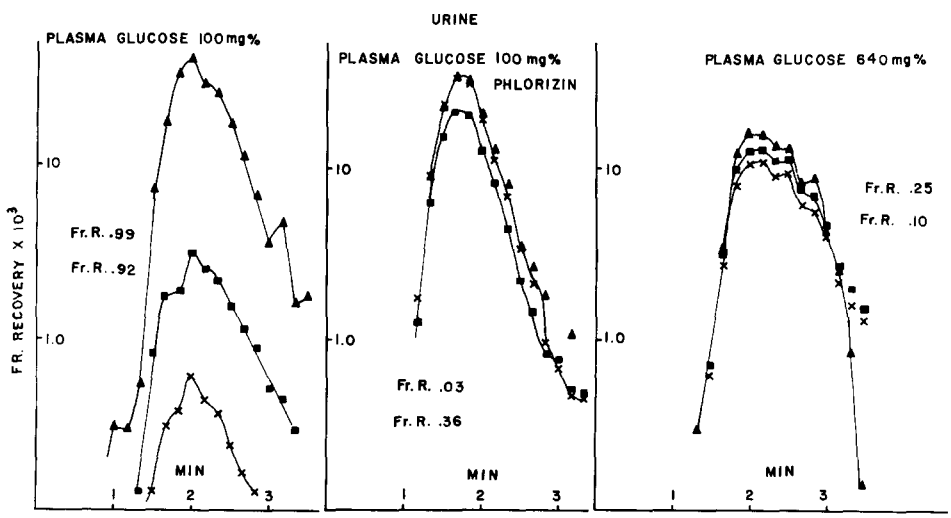

Fig. 9. Urine outflow curves for creatinine (▲), D-[^{14}C]glucose (×), and [^{3}H]*myo*-inositol (■), under control conditions (left-hand panel), phlorizin loading (middle panel), and glucose loading (right-hand panel).

[^{14}C]glucose and [^{3}H]*myo*-inositol. In the presence of phlorizin (middle panel Fig. 9) the reabsorption of tracer glucose is completely inhibited as evidenced by the superposition of the glucose and creatinine curves. Despite the blockage of the G receptors, 36% of *myo*-inositol is still extracted by the luminal membrane. Raising the plasma glucose concentration from 100 to 640 mg/100 ml (right-hand panel Fig. 9) reduces the fractional reabsorption of *myo*-inositol almost to zero. Thus glucose is a more effective inhibitor of *myo*-inositol reabsorption than phlorizin. To explain this data we might postulate that there is something special about the interaction of glucose with the glucose transport mechanism which does not occur when phlorizin binds to the same G receptor site. Our results are therefore consistent with the data of Caspary and Crane [19] who feel that the interference of glucose with *myo*-inositol transport in hamster intestine occurs at the level of the "second step" in the glucose transport process (which could include the translocation phase), rather than with the postulated binding phase.

Mannose loading has no effect on glucose or *myo*-inositol interaction with the luminal membrane. Thus it is entirely possible that a separate set of "*myo*-inositol' specific receptor sites (independent of phlorizin but accessible to glucose) exists along the brush border of the proximal tubule and that these are distinct from both the G and M receptor sites previously defined.

X-ray diffraction patterns of *myo*-inositol show that the OH group at the carbon 2 position is axial to the mean plane of the pyranose ring [20]. Inspection of CPK molecular models for *myo*-inositol and glucose reveals that the requirements for OH groups on Carbon 3 and 6 of D-glucose could be satisfied by two pairs of OH groups on *myo*-inositol (C4–C2, C3–C2). The distance separating these two OH groups is somewhat less in the case of *myo*-inositol than the equivalent distance in D-glucose. By inspection of space-filling molecular models and the experimental finding showing that *myo*-inositol shares the G sites we are again made aware of the importance of the steric arrangement of certain "key" functional OH groups relative to the pyranose ring in determining the interaction with glucose transport receptors. Moreover, the oxygen in the pyranose ring does not seem to play a crucial role in the monosaccharide–receptor interaction. Therefore the minimal structural requirement may be a cyclohexane-like ring with certain key functional hydroxyl substituents. Admittedly there is free rotation around the C5–C6 bond, and the axial position of the OH group on carbon 6 as shown in Fig. 8 for D-glucose might not represent the most stable form in aqueous solution. However if we try to look for common structural features to explain the sharing of the same transport receptor for glucose, *myo*-inositol and fructose then our data suggest that the presence of an electronegative OH group roughly perpendicular to the mean plane of the pyranoside (i.e. on carbon 6 of D-glucose and carbon 2 of *myo*-inositol) may play a crucial role in the dynamics of the transport of sugars (monosaccharides) across the brush border.

DISCUSSION

Our present results are consistent with the following chemical and stereospecificity requirements governing sugar interactions with renal tubular surfaces.

(a) Luminal. (1) A pyranose ring in a chair conformation (C1 or 1C); (2) OH

groups on carbons 3 and 6 oriented as in the configuration of D-glucose.

(b) Antiluminal. (1) A pyranose ring in a chair conformation (C1 or 1C); (2) hydroxyl groups on carbons 1 and 2; (3) OH groups if present on carbons 3 and 6 should be oriented equatorially as in the configuration of D-glucose.

It must be emphasized that the specificity characteristics of sugar interaction with the antiluminal membrane might represent several different receptors. Further investigation with competitive inhibition studies is required to settle this question.

The cumulative data on the specificity of sugar reabsorption across the luminal membrane point to the existence of two and possibly three sets of membrane receptors for monosaccharides. The crucial question is whether only one or all three of these receptors are involved in sugar reabsorption. Of the 23 pyranoses tested in our series, (ref. 2 and present study), only 8 have been found to have any observable interaction with the brush border of the proximal tubule. Seven of the eight pyranosides share a phlorizin-sensitive receptor with D-glucose. The existence of an alternate pathway for *myo*-inositol other than the G or M receptor is also possible. However there is no experimental evidence to suggest that mannose or *myo*-inositol receptors are involved in transmembrane transport of sugars. Instead it is entirely possible that certain glycoprotein binding sites located on the membrane can bind a fraction of tracer mannose or *myo*-inositol and incorporate these labels into their structure.

It is interesting to compare our results in vivo with the in vitro results of Kleinzeller using cortical slices [21, 22]. Although slices do not permit distinction of luminal from antiluminal events, the data show that there is a transport system for D-glucose which is (i) sodium-dependent, (ii) phlorizin-sensitive, and (iii) capable of accumulating glucose against a concentration gradient. The specificity of this transport system consists of a pyranose ring in the Cl chair conformation and D-configuration and the presence of OH groups on carbon 3 and 6. Thus Kleinzeller's active transport system for D-glucose in the rabbit has identical specificity characteristics to our G transport receptor on the luminal membrane of the proximal tubule in dog kidney.

The recent studies of Glossmann and Neville, Jr [4] as well as Bode et al. [5] also point out remarkable similarities between the G transport sites in the dog and a set of high affinity phlorizin binding sites in membrane fractions prepared from rat kidney. Except for some minor differences, (i.e. the behaviour of D-fructose) the specificity characteristics of the G transport receptor in the dog match up with those of the phlorizin-sensitive receptor sites in the rat. In particular D-glucose, D-galactose, 2-deoxy-D-glucose, methyl-α- and β-D-glucoside all inhibit binding.

On the basis of the previous considerations we are left with the tempting conclusion that in the rat, rabbit, and dog kidney there exist a unique, homogeneous set of receptor sites for the transport of D-glucose: the so-called G transport receptors, and that these transport receptors have distinct specificity characteristics (given above).

There are obvious pitfalls in trying to interpret the significance of sugar–membrane interactions in vitro using only isolated membrane preparations. One of the purposes of the foregoing discussion is to emphasize the importance of having a back-up reference of identifying the presence of the true (in vivo) transport system system for sugars. In particular we have tried to indicate how the in vivo chemical

and stereospecificity data may serve as a "monitor" for binding assays of sugar transport receptors performed on intact or solubilized membrane preparations.

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