Tubular reabsorption of myo-inositol vs. that of D-glucose in rat kidney in vivo et situ

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Tubular reabsorption of myo-inositol vs. that of D-glucose in rat kidney in vivo et situ. Am J Physiol Renal Physiol 284: F1181–F1189, 2003; 10.1152/ajprenal.00395.2002.—Filtered myo-inositol, an important renal intracellular organic osmolyte, is almost completely reabsorbed. To examine tubule sites and specificity and, thus possible mechanism of this reabsorption, we microinfused myo-[3H]inositol or D-[3H]glucose into early proximal (EP), late proximal (LP), or early distal tubule sections of superficial nephrons and into long loops of Henle (LLH) of juxtamedullary nephrons and papillary vasa recta in rats in vivo et situ and determined urinary fractional recovery of the [3H] label compared with comicroinfused [14C]inulin. To determine the extent to which the proximal convoluted tubule (PCT) alone contributes to myo-inositol reabsorption, we also microperfused this tubule segment between EP and LP puncture sites. We examined specificity of reabsorptive carrier(s) by adding high concentrations of other polyols and monosaccharides to the infusion. The results show that >60% of the physiological glomerular load of myo-inositol can be reabsorbed in the PCT and >90% in the short loop of Henle (SLH) by a saturable, phlorizin-sensitive process. myo-Inositol can also be reabsorbed in the ascending limb of LLH and can move from papillary vasa recta blood into ipsilateral tubular structures. Essentially no reabsorption occurred in nephron segments beyond the SLH or in collecting ducts. Specificity studies indicate that reabsorption probably occurs via a luminal Na+–myo-inositol cotransporter.

myo-inositol transport; D-glucose transport; sodium–myo-inositol cotransporter

MYO-INOSITOL (Mr = 180) has at least two important functions in mammalian cells. First, it is a phosphoinositide precursor and, therefore, plays an integral role in phospholipase C-mediated and other signal transduction pathways. Second, it is an intracellular organic osmolyte in the kidney (especially in the outer medulla) and several other tissues (12, 13, 17, 18, 20, 26, 37, 38). In rats, myo-inositol plasma concentration amounts to ~50 μmol/l, and myo-inositol concentrations of >20, 15, and <5 mmol/l have been found in cell water in rat thick ascending limbs, collecting ducts, and proximal tubules, respectively (23). Similar values have been reported for the rabbit kidney (37), where the concentration of myo-inositol in the outer medulla decreased from ~35 (in antidiuresis) to ~25 mmol/kg wet wt (in water diuresis). The high myo-inositol concentration gradient between cytosol and extracellular fluid is maintained by a Na+–myo-inositol cotransporter (SMIT) (15). In collecting duct-derived Madin-Darby canine kidney (MDCK) cells (21a), a hypertonic environment induced an increased transcription of the SMIT gene and, at the same time, a dramatically increased cytosolic myo-inositol concentration (34, 36). Of this uptake, 90% occurred at the basolateral cell side (34). In rat kidney, SMIT was found to be strongly expressed in the medulla and, at lower levels, in the cortex (35). In situ hybridization revealed that SMIT is predominantly present in the medullary and cortical thick ascending limbs of Henle’s loop and macula densa cells (35).

Fractional excretion of myo-inositol by the rat kidney amounts to 1–2% (6). Thus highly effective transporter(s) must exist in the luminal membrane of the renal tubules. Takenawa et al. (28) investigated myo-inositol transport in a cortical plasma membrane preparation of rat kidney. They found an uptake mechanism specific for myo-inositol and scyllitol that did not accept D-glucose or D-galactose. In a similar study, Hammerman et al. (11) examined myo-inositol uptake into rabbit renal cortical brush-border membrane vesicles. They found that myo-inositol uptake was electrogenic and saturable as well as stimulated by a electrochemical Na+ gradient. Uptake was inhibited by phlorizin and, to a moderate extent, by D-glucose.

In the present study, we investigated the localization and kinetics of tubular myo-inositol reabsorption in rat kidney and characterized the specificity of the transport mechanism to identify the carrier type(s) involved in tubular myo-inositol reabsorption. For this purpose, we microinfused myo-inositol or D-[3H]glucose into early proximal (EP), late proximal (LP), or early distal (ED) tubule sections of superficial nephrons as well as into long loops of Henle (LLH) of juxtamedullary nephrons of the rat in vivo et situ and determined the fractional recovery of the [3H] label compared with comicroinfused [14C]inulin in the final urine. To determine the extent to which the proximal convoluted tubule...
(PCT) alone contributes to myo-inositol reabsorption, we also microperfused this tubule segment between EP and LP puncture sites. For any reabsorption of myo-[\textsuperscript{3}H]inositol or d-[\textsuperscript{3}H]glucose found, we then examined the specificity of the carrier(s) involved by adding high concentrations of other polyols and monosaccharides to the infusate or perfusate. To elucidate whether myo-inositol in medullary plasma has access to the ipsilateral lumen of collecting ducts, we also microinfused myo-[\textsuperscript{3}H]inositol or d-[\textsuperscript{3}H]glucose together with [\textsuperscript{14}C]inulin into ascending vasa recta and determined the fractional recovery of the \textsuperscript{3}H label (compared with microinfused [\textsuperscript{14}C]inulin) in the ipsilateral and contralateral urine.

MATERIALS AND METHODS

Male Munich-Wistar rats were used in the following three experimental groups: group A (EP, LP, and ED experiments; see Figs. 2–4, 6, and 7; mean body weight 178–320 g (mean wt: 270 g), purchased from Simonsen Laboratories, Gilroy, CA; group B (PCT microinfusion experiments; see Figs. 8 and 9; body weight 192–475 g (mean wt: 366 g), purchased from Medizinische Hochschule, Hannover, Germany); and group C (LLH experiments; body weight 61–146 g (mean wt: 99 g), purchased from Medizinische Hochschule). Group A was fed on Teklad 4% Mouse/Rat Diet 7001; groups B and C were fed on an Altromin Standard Diet 1320. All groups had free access to water. The animals were anesthetized with Inactin (Byk-Gulden, Konstanz, Germany; 120 mg/kg body wt). A tracheostomy was performed, and polyethylene cannulas were placed in the right jugular vein for infusions. The animals were infused with Ringer solution at a rate of 0.05 ml/min kg body wt for the larger animals (groups A and B) and 0.02 ml/min kg body wt for the smaller animals (group C). The Ringer contained the following (in g/l): 9 NaCl, 0.4 KCl, 0.25 CaCl\textsubscript{2}, and 0.2 NaHCO\textsubscript{3}. The kidney was collected from a bladder catheter in 30-min fractions for 1 h, and the \textsuperscript{3}H ([\textsuperscript{3}H]urine) and \textsuperscript{14}C ([\textsuperscript{14}C]urine) disintegrations/min (dpm) were counted. For details, see MATERIALS AND METHODS.

Microinfusion into superficial nephrons (EP, LP, ED). After identification of the nephron segments by intravenous injection of lissamine green SF (Chroma-Gesellschaft, Schmidt, König, Germany) at a dose of 0.02 ml of a 100-g/l solution titrated with NaOH to pH 7.4, the tubule was micropunctured using glass capillaries (see Fig. 1). The latter had ground tips (outer tip diameter 9\textmu m) and were mounted on a microperfusion pump (25). Microinfusion sites were (see Fig. 1) 1) the first superficial loop of the proximal tubule (EP), 2) the last superficial loop of the proximal tubule (LP), and 3) the first superficial loop of the distal tubule (ED). In all these cases, the microinfusate (10 nl/min) added to the endogenous flow rate of tubular fluid. The microinfusate (pH 6.7) contained (in mmol/l) 154 NaCl, 5.4 KCl, 1.7 CaCl\textsubscript{2}, 9.6 MOPS, 88 MBr/g (= 2.4 mCi/g) [\textsuperscript{14}C]inulin (NEN, Perkin-Elmer Life Science, Boston, MA); 10 mmol/l \textsuperscript{3}H-labeled myo-inositol (2.04 GBq = 55 mCi/\textmu mol, American Radiolabeled Chemicals, St. Louis, MO), or 10 mmol/l \textsuperscript{3}H-labeled D-glucose (2.23 GBq/\textmu mol = 60 mCi/\textmu mol, American Radiolabeled Chemicals), as well as unlabeled sugars, polyols, or polyhydridin, as indicated in RESULTS (see Figs. 6 and 7). Microinfusion lasted for 10 min. Starting shortly before microinfusion, the ipsilateral ureter was collected from the ureteral catheter in 30-min fractions for 1 h, and the \textsuperscript{14}C and \textsuperscript{3}H disintegrations per minute (dpm) counts of each fraction were determined in a liquid scintillation spectrometer (Beckman LS 6000SE, Anaheim, CA, or Canberra-Packard 1600 TR, Frankfurt/Main, Germany). As a control, the urine of the contralateral kidney was collected from a bladder catheter in 30-min fractions during the same 1-h period. The [\textsuperscript{14}C]inulin counts (if any) and the \textsuperscript{3}H counts in the contralateral urine, never exceeding 10% of those in the ipsilateral urine, were subtracted from the latter. After this correction, the fractional recovery (see Fig. 1) was calculated from the sum of the \textsuperscript{14}C and \textsuperscript{3}H dpm counts, respectively, of the 1-h collecting period ([\textsuperscript{14}C]urine and \textsuperscript{3}H]urine; see Fig. 1) and from the \textsuperscript{14}C and \textsuperscript{3}H dpm counts of the microinfusion solution ([\textsuperscript{14}C]inf and \textsuperscript{3}Hinf; see Fig. 1). For the latter purpose, the 10-min output of the microinfusion pump was collected in a drop of water.

Microinfusion into LLH. The experiments on LLH were performed as described previously (4, 5). Briefly, the papilla of the left kidney was exposed, and a single ascending limb of a LLH was punctured near the hairpin bend with a glass micropuncture pipette, having an external tip diameter of 5–6 \textmu m, and mounted to a microperfusion pump (25). The tip of this pipette was coated with platinum glaze to make it easily visible (9). The loop was then infused with a solution containing [\textsuperscript{14}C]inulin and myo-[\textsuperscript{3}H]inositol (90 \textmu M) or d-[\textsuperscript{3}H]glucose (35 \textmu mol/l) as described above as well as (in mmol/l) 154 NaCl, 5.4 KCl, 1.7 CaCl\textsubscript{2}, 2.4 NaHCO\textsubscript{3}, and 10 TES. The microinfusion solution also contained lissamine green (20 g/l) so that the flow in the loop could be seen and we could determine whether there was any extravasation from the loop that would make the infusion technically unacceptable. The microinfusion was generally maintained at 10 nl/min. After the microinfusion was well established (usually 2–3 min), collections of urine emerging from the ducts of Bellini were made with a second micropuncture pipette (external tip diameter 12–14 \textmu m) (see Fig. 1). The urinary volume recovery at this collection site was much smaller than...
that obtained from the ureteral catheter during microinfusion into superficial nephrons. Therefore, the lowest myo-[3H]inositol concentration used had to be higher (90 μmol/l) than that used for superficial nephrons (10 μmol/l). The same consideration applied to the concentration of D-[3H]glucose (33 vs. 10 μM). The radioactivity in the collected fluid and the initial perfusion solution was measured in a liquid scintillation counter (1600 TR, Canberra-Packard) to determine the fractional recovery of the infused myo-[3H]inositol in the urine of the ducts of Bellini. Two to five collections of ~70–100 nl were made in each infusion experiment (the number depending on the length of time the infusion could be maintained), and the mean value for the fractional recovery for all collections was used as the value for that microinfusion experiment.

Microinfusion into ascending vasa recta. The infusions into the ascending vasa recta (AVR) were performed in a manner identical to that described above for infusions into ascending loops of Henle. AVR were easily indentified by observing the direction of flow of the red blood cells. As in our previous study (4), during the constant infusion of a vas rectum, we collected urine simultaneously with a microdialysis probe from the first exposed papilla, as described above, and from the contralateral kidney via the bladder cannula. Because it is relatively easy to puncture and infuse an ascending vas rectum, we could maintain the infusion long enough, as in our previous experiments (4), for the inulin infused to be uniformly distributed and filtered by both kidneys. Within the first 5 min, inulin appeared to be uniformly distributed in the animal (i.e., a steady state appeared to be attained) so that the amounts obtained from the ipsilateral and contralateral kidneys were equal (4). They remained equal as long as the constant infusion was maintained. Also, once this point was attained, the amounts of the other substance infused (e.g., myo-inositol) collected from each kidney remained constant over time as long as the constant infusion was maintained (4). The usual length of these infusions was 20–25 min.

In determining the amount of myo-inositol or D-glucose relative to inulin appearing in the collections from each kidney (see also Ref. 4), we took into account the fact that at a steady state (assuming equal glomerular filtration rates of both kidneys) one-half of the infused inulin should be excreted by each kidney. Thus we divided by two the quotient of the myo-inositol-to-inulin ratios in the urine vs. infusate ([myo-inositol]/[inulin] urine/([myo-inositol]/[inulin] infusate). This gives the fraction of microinfused myo-inositol (relative to inulin) excreted on each side. The sum of the fractions for the ipsilateral and contralateral kidneys gives the total fraction of the infused myo-inositol (relative to inulin) excreted by both kidneys combined. The difference between the values obtained for the ipsilateral and contralateral kidneys gives the fraction of the microinfused myo-inositol (relative to inulin) secreted on the ipsilateral side. The same approach was used for D-glucose microinfusions into the AVR. All other aspects of the infusions, as well as the collection of urine emerging from the ducts of Bellini and the handling of samples, were as described above for infusions into the LLH.

Microperfusion of the PCT. Segments of PCT were microperfused (25) at a rate of 20 nl/min with the following solution (in g/l) 6.2 NaCl, 0.37 KCl, 0.22 CaCl2, and 2.2 TES and titrated to pH 7.4. For this purpose, Sudan black-stained fat was microinfused with a microcatheter into the first superficial loop of the proximal convolution, and the endogenous tubule fluid was drained subsequently into the same pipette. The tubule was microperfused with a second microcatheter between the second superficial loop (distal end of the oil block) and the last accessible loop of the proximal convolution (perfusion length ~2–3 mm), where the perfusate was collected and the fractional late proximal recovery of myo-[3H]inositol or D-[3H]glucose was determined.

Calculations and statistics. The maximal reabsorption rate Jmax (pmol/s) and the apparent Michaelis constant (mmol/l) of myo-[3H]inositol reabsorption from short loops of Henle were obtained by fitting the Michaelis-Menten equation J = Jmax·Clp/(Km + Clp), whereby J = (1 – fractional recovery)·microinfusion rate·Cm (pmol·s−1·short loop of Henle−1).

Because there was virtually no myo-[3H]inositol reabsorption downstream of the ED microinfusion site (see RESULTS), fractional reabsorption during LP microinfusion is equivalent to that in short loops of Henle. All results shown in Figs. 2–4 and 6–9 are means ± SE (n = no. of microinfused or microperfused nephron segments). The level of significance for differences between means of unpaired observations was determined with Student’s t-test. Differences assessed by t-test were considered statistically significant at P < 0.05.

RESULTS

Because we wanted to find out where along the nephron and to what extent myo-inositol is reabsorbed, we microinfused a solution containing myo-[3H]inositol and [3H]ulin into superficial tubular puncture sites and determined the fractional recovery (see MATERIALS and METHODS for details). The fractional reabsorption in the different segments of the nephron is shown in Fig. 2. Fractional 3H recovery in the final urine (in %) during microinfusion (10 nl/min) of 0.01, 1, 3, 10, or 50 mmol/l myo-[3H]inositol into EP, LP, and ED segments of superficial nephrons. Values are means ± SE; the no. of microinfused nephron segments is in parentheses.
Fig. 3. Fractional 3H reabsorption (=100 – fractional recovery of 3H in the ipsilateral final urine (in %)] during microinfusion (10 nl/min) of 90 μmol/l myo-[3H]inositol into long loops of Henle in the presence or absence of 0.1 mmol/l phloridzin or 50 mmol/l nonlabeled myo-inositol. Values are means ± SE; the no. of microinfused nephron segments is in parentheses.

Localization, saturation, phloridzin sensitivity, and pH dependence of myo-inositol reabsorption along the nephron. In a first series of experiments, we microinfused (10 nl/min) a solution containing 10 μmol/l 3H-labeled myo-inositol into EP, LP and ED tubule sections appearing at the surface of the kidney. As can be seen from Fig. 2, fractional reabsorption of the 3H label of myo-inositol was 95 ± 1.4% during EP, 96 ± 0.8% during LP, but not significantly different from zero (3.7 ± 5.2%, n = 7) during ED microinfusion. Thus regardless of whether myo-[3H]inositol was injected at EP or LP sites, reabsorption was virtually complete. In contrast, no reabsorption at all was observed between the ED microinfusion site of these superficial (short) nephrons and the final ipsilateral urine. Phloridzin (0.1 mmol/l) added to the microinfusate nearly completely blocked reabsorption of 10 μmol/l myo-[3H]inositol during EP and LP microinfusion. All these microinjection solutions were buffered to a pH of 6.7. To test whether a higher pH influences the rate of reabsorption, we repeated the LP experiment with 10 μmol/l myo-[3H]inositol at pH 7.6. However, the fractional reabsorption (96 ± 0.31%, n = 8) did not change at all.

Next, we increased the myo-inositol concentration in the microinfusate to 1, 3, 10 and 50 mmol/l (pH 6.7) by adding the nonlabeled compound. As shown in Fig. 2, fractional reabsorption decreased more as the concentration was increased; i.e., transport became saturated. Whereas at 10 μmol/l and 1 mmol/l, fractional reabsorption was found to have nearly the same high value at EP and LP microinfusion sites, at 3 mmol/l, fractional reabsorption during LP microinfusion was only 60% of that during EP microinfusion. At 50 mmol/l, fractional reabsorption amounted to ~15%; i.e., it became nearly fully saturated. As expected, fractional reabsorption during ED microinfusion was still not significantly different from zero at myo-inositol concentrations of 1 (7.12 ± 2.45%, n = 4) and 10 mmol/l (4.99 ± 2.45, n = 4). At 50 mmol/l myo-inositol, the significance level was just reached (P = 0.05). These data demonstrate that myo-[3H]inositol reabsorption from the loop of Henle is mediated by a high-capacity transporter. Apparent kinetic parameters roughly estimated from the LP values of Fig. 2 were as follows: J_{max} = 0.88 ± 0.14 pmol·s⁻¹·short loop of Henle⁻¹; K_{m} (concentration at ½J_{max}) = 3.4 ± 1.67 mmol/l (n = 36).

When we microinfused a solution containing 90 μmol/l 3H-labeled myo-inositol plus [14C]inulin into the ascending limb of an LLH near the hairpin bend, 44 ± 4.6% (n = 15) of myo-[3H]inositol was reabsorbed between the puncture site and the urine emerging from the ipsilateral ducts of Bellini. This fraction did not change when 0.1 mmol/l phloridzin or 50 mmol/l nonlabeled myo-inositol was added to the microinfusate (see Fig. 3). Thus myo-inositol is able to leave the lumen of tubule segments that are situated downstream from the LLH microinfusion site. Because ED microinfusion of myo-[3H]inositol did not result in any reabsorption (see Fig. 2), the collecting duct is not involved in the reabsorptive process. Thus the myo-[3H]inositol microinfused into LLH must have been reabsorbed in the ascending limb of Henle’s loop of juxtamedullary nephrons.

Microinfusing the same solution (90 μmol/l myo-[3H]inositol plus [14C]inulin) into vasa recta running parallel to the LLH, we found that fractional recovery was 27 ± 0.05% (n = 8) in the ipsilateral urine and 1.8 ± 0.007% (n = 7) in the contralateral urine. The
former value decreased significantly to 12 ± 0.02% (n = 7) (and the contralateral one did not change) when 0.1 mmol/l phloridzin or 50 mmol/l nonlabeled myo-inositol was added to the microinfusate (see Fig. 4). Thus myo-[3H]inositol is also able to enter the tubular urine from the ipsilateral vasa recta blood in a phloridzin-sensitive and saturable manner.

Localization, saturation, and phloridzin dependence of D-glucose reabsorption along the nephron. To obtain insight into the molecular specificity of myo-inositol vs. D-glucose reabsorption in the loop of Henle (see below), we first had to characterize D-glucose reabsorption in experiments similar to those used with myo-inositol.

First, we determined the fractional reabsorption of 10 μmol/l 3H-labeled D-glucose during EP, LP, and ED microinfusion and obtained the following values: 88.7 ± 2.2% (EP, n = 5), 91.3 ± 2.6% (LP, n = 6), and 51.1 ± 0.4% (ED, n = 6). Thus the high fractional reabsorption of D-glucose determined during LP microinfusion reflects nearly exclusively reabsorption in short loops of Henle, because reabsorption beyond the ED site is very small. At higher initial D-glucose concentrations in the tubule segments that are situated downstream from the LLH microinfusion site, 31.0 ± 8% (n = 10) of D-[3H]glucose was reabsorbed between the puncture site and the urine emerging from the ipsilateral ducts of Bellini. This fraction did not change significantly when 0.1 mmol/l phloridzin or 50 mmol/l nonlabeled D-glucose was added to the microinfusate (see Fig. 3). Thus D-glucose is able to leave the luminal side of the nephron’s segments that are situated downstream from the LLH microinfusion site. As ED microinfusion of D-[3H]glucose resulted only in a very small fractional reabsorption, the collecting duct does not seem to be involved in the reabsorptive process. Thus the D-[3H]glucose microinfused into LLH must have been reabsorbed in the ascending limb of Henle’s loop of juxtedudillary nephrons.

Microinfusing the same solution (33 μmol/l D-[3H]glucose plus [14C]inulin into vasa recta running parallel to the LLH, we found that fractional recovery was 25 ± 4% (n = 4) in the ipsilateral urine and 5 ± 1% (n = 4) in the contralateral urine. These values did not change significantly when 0.1 mmol/l phloridzin or 50 mmol/l nonlabeled D-glucose was added to the microinfusate. Thus D-[3H]glucose is also able to enter the tubular urine from the ipsilateral vasa recta blood.

Molecular specificity of myo-inositol vs. D-glucose reabsorption in the loop of Henle. In further sets of experiments, we evaluated the molecular specificity of the carrier(s) involved in tubular myo-[3H]inositol reabsorption during LP microinfusion. For this purpose, 50 mmol/l of the following compounds were added to the microinfusate containing 10 μmol/l 3H-labeled myo-inositol plus [14C]inulin: nonlabeled myo-inositol, scyllo-inositol, D-chiro-inositol, L-chiro-inositol (see Fig. 5), D-fructose, D-mannose, L-fructose (6-deoxy-L-galactose), 3-O-methyl-galactose (=3-O-methyl-D-glucopyranose), D-glucose, D-galactose, and α-methyl-D-glucoside. As can be seen from Fig. 6, scyllo-inositol and D-chiro-inositol had nearly the same large inhibitory effect as nonlabeled myo-inositol itself, whereas L-chiro-inositol, D-glucose, D-galactose, and α-methyl-D-glucoside had a much smaller but significant inhibitory effect on myo-[3H]inositol reabsorption. No inhibition occurred in the presence of the remaining four compounds.

The small effect of D-glucose on the reabsorption of myo-inositol (Fig. 6) could have meant that myo-inositol is transported by one of the D-glucose transporters (SGLT1 and/or 2) at a significantly higher affinity than D-glucose itself. Therefore, we tested the extent to which myo-inositol and the strong inhibitor of its reabsorption, D-chiro-inositol (see Fig. 6), as well as other sugars and polyols influenced reabsorption of D-glucose. For this purpose, we microinfused 10 μmol/l of the carrier(s) involved in tubular myo-[3H]inositol reabsorption during LP microinfusion.
The individual compounds (50 mmol/l, except for phloridzin) were added (+) to the microinfusion solution. Values are means ± SE; the no. of microinfused nephron segments is in parentheses.

D-[^3]Hglucose (plus [14C]inulin) at LP microinjection sites in the presence of 50 mmol/l of nonlabeled myo-inositol, d-chiro-inositol, l-chiro-inositol, d-fructose, l-fucose, 3-O-methylglucose, d-galactose, and α-methyl-D-glucoside. The results are depicted in Fig. 7. Whereas the reabsorption of D-glucose was 91.3% at a concentration of 10 μmol/l (control) and was reduced to 35.5% at 50 mmol/l, it was inhibited to only a moderate or small extent by the addition of 50 mmol/l of nonlabeled D-fructose, α-methyl-D-glucoside, or D-galactose. Fractional reabsorption of D-glucose in the presence of these three compounds was 62.8 ± 9.4, 66.4 ± 4.9, and 81.9 ± 5.6%, respectively. At 50 mmol/l myo-inositol, 3-O-methylglucose (=3-O-methyl-D-glucopyranose), d-chiro-inositol, l-chiro-inositol, and l-fucose (=6-deoxy-l-galactose) did not have any significant effect on D-[^3]Hglucose reabsorption (Fig. 7).

Reabsorption of myo-inositol in the PCT. As can be seen from Fig. 2, fractional reabsorption of myo-inositol at an initial concentration of 1 and 3 mmol/l was higher during EP than during LP microinfusion. This means that the tubule segment located between these two microinfusion sites, i.e., the PCT, contributes to renal myo-inositol reabsorption. To study this process in more detail directly, we microperfused the segment between EP and LP micropuncture sites with a solution containing 10 μmol/l 3H-labeled myo-inositol (+ [14C]inulin) in the absence and presence of 0.1 mmol/l phloridzin or 50 mmol/l nonlabeled myo-inositol, D-glucose, L-fucose, and 3-O-methylglucose at a microperfusion rate of 20 nl/min. The fractional recovery of the 3H activity was determined in the perfusate collected at LP micropuncture sites. As shown in Fig. 8, fractional reabsorption of 10 μmol/l myo-inositol was 63.3 ± 3.7% (control) and decreased to 9.2 ± 2.3, 36.3 ± 2.2, or 17.3 ± 2.4% in the presence of 50 mmol/l of nonlabeled myo-inositol, D-glucose, or 0.1 mmol/l phloridzin, respectively. L-Fucose and 3-O-methylglucose did not have a significant effect. Qualitatively, these results resemble those for the short loops of Henle (see Fig. 6), but fractional reabsorption was generally lower in the microperfused segment of the proximal convoluted than that in short loops of Henle. The microperfusion solution had a higher pH than that of the LP microinfusion experiments (see MATERIALS AND METHODS). However, this cannot be the reason for the quantitative difference in reabsorption, because myo-inositol reabsorption was pH independent in this pH range.

Reabsorption of D-glucose in the PCT. In additional EP microinfusion experiments, we found that frac-
tional reabsorption of D-glucose at an initial concentration of 50 mmol/l (70.1 ± 4.0%, n = 5) was much higher than during the LP microinfusion reported above (35.5 ± 4.8%, n = 5). This last finding was not unexpected and indicates that the PCT contributes a major part to renal D-glucose reabsorption. To test the influence of myo-inositol and other compounds on proximal D-glucose reabsorption directly, we microperfused the segment between EP and LP micropuncture sites with a solution containing 10 μmol/l [3H]-labeled D-glucose (+[14C]-inulin) in the absence and presence of 0.1 and 1.0 mmol/l phloridzin or 50 mmol/l nonlabeled D-glucose, myo-inositol, L-fucose, or 3-O-methylglucose at a microperfusion rate of 20 nl/min. As shown in Fig. 9, fractional reabsorption of 10 μmol/l D-glucose was 74.1 ± 2.8% (control) and decreased to 15.7 ± 2.5% in the presence of 50 mmol/l nonlabeled D-glucose and to 16.5 ± 1.4 and 8.0 ± 3.3% in the presence of 0.1 and 1 mmol/l phloridzin, respectively. L-Fucose and 3-O-methylglucose had a small effect, whereas 50 mmol/l myo-inositol did not influence D-glucose reabsorption at all. Qualitatively, these results again mirror those from the short loops of Henle (see Fig. 7), but fractional reabsorption is generally lower in the microperfused segment of the proximal convolution than in the short loops of Henle.

DISCUSSION

It has been known for about 50 years that myo-inositol is nearly completely reabsorbed in the mammalian kidney (6). However, only very little has been known about the localization of the reabsorptive process along the nephron and the transport mechanism and its molecular specificity. We investigated these topics by microinfusing and microperfusing single tubule segments of rat kidney in vivo and in situ. Our results indicate that >60% of the physiological glomerular load of myo-inositol can be reabsorbed in the PCT. Moreover, the short loop of Henle alone was able to reabsorb >90% of a myo-inositol load that is higher than the physiological glomerular load. In both segments, myo-inositol reabsorption was phloridzin sensitive and saturable. These findings are in accord with earlier in vitro results showing that renal cortical brush-border membrane vesicles (BBMV) from rat and rabbit take up myo-inositol by a saturable, phloridzin-sensitive process that is stimulated by an electrochemical Na⁺ gradient (11, 27).

Essentially, no myo-inositol reabsorption occurred in nephron segments beyond the short loops of Henle and in the collecting ducts. This means that reabsorptive data obtained during LP microinfusions represent reabsorption in short loops of Henle. Our data obtained by microinfusing the ascending limb of LLH revealed that about one-half of the microinfused myo-[3H]inositol was reabsorbed downstream from this micropuncture site. The reabsorption must take place in the ascending limb of LLH because the collecting ducts are not able to reabsorb myo-inositol (see above). Moreover, myo-[3H]inositol microinfused into inner medullary AVR appeared in the ipsilateral final urine to a much greater extent than in the contralateral urine. This transport out of the vasa recta was saturable and sensitive to phloridzin. These results taken together suggest that myo-inositol is able to enter medullary cells from the blood side as well as from the tubular lumen. We cannot exclude the possibility that myo-inositol could be secreted in the connecting tubule in the medullary ray, but we do not think that it is likely.

In these studies, we have shown that myo-inositol reabsorption in the short loops of Henle and in the PCT is nearly completely saturated if a high concentration of nonlabeled myo-inositol is added to the microinfusate. To test the specificity of this transport and, at the same time, to get an idea of which apical carrier(s) is involved in tubular myo-inositol reabsorption, we tried to inhibit it with high concentrations of several polyols and sugars. We observed a strong inhibition of myo-inositol reabsorption when it was infused together with the myo-inositol derivatives scyllo-inositol (or scyllitol) or D-chiro-inositol. Scyllo-inositol has previously been shown to inhibit strongly myo-inositol uptake into rat BBMV (27). Moreover, scyllo-inositol induces nearly the same steady-state current as myo-inositol in oocytes into which cRNA of canine SMIT has been injected (10).

As illustrated in Fig. 6, D-fructose, D-mannose, L-fucose, and 3-O-methylglucose did not have any effect on myo-inositol reabsorption. Thus it is very unlikely that myo-inositol is reabsorbed by the fructose uniporter GLUT5 or a mannose symporter (7, 24).

We tested L-fucose and 3-O-methylglucose because previous studies have shown that uptake currents in canine SMIT-injected oocytes are high with L-fucose but very low with 3-O-methylglucose (10). In the same paper, uptake current was also measured in oocytes...
transferred with rabbit Na+-glucose luminal transporter 1 (SGLT1). In these cells, 3-O-methylglucose was very well accepted by SGLT1 but L-fucose not at all (10). Our results, i.e., that neither of the two substances influenced myo-inositol transport in the short loop of Henle (Fig. 6) or the PCT (Fig. 8), could mean that myo-inositol is not reabsorbed by either SMIT or SGLT1. However, although SMIT from the dog and SMT from the rat have a 95% homology at the protein level (358 amino acids in both), dog cDNA (2,870 bases) is much longer than rat cDNA (1,155 bases) (15, 17, 35). Therefore, the specificity of canine SMIT might be quite different from that of the rat because splice variants of SMIT have been found even in the same species (8, 22). Thus we cannot rule out the possibility that SMIT is responsible for the reabsorption of myo-inositol in the rat kidney.

However, are the D-glucose transporters in tubule apical membranes involved in myo-inositol reabsorption? Tubular D-glucose reabsorption shows axial heterogeneity (29). SGLT1 is present in the S3 segment and SGLT2, in the S1 and S2 segments of the proximal tubule (14, 16). Substrates of the rat and rabbit SGLT1 are D-glucose, α-methyl-D-glucoside, D-galactose, and 3-O-methyl-D-glucoside (10, 16); substrates of the rabbit and pig SGLT2 are D-glucose and α-methyl-D-glucoside, but not D-galactose and 3-O-methyl-D-glucose (14, 19). However, localization and/or specificity of these transporters does not seem to be clear cut because D-galactose is reabsorbed in the PCT (32), which does not include the S3 segment. In the present study, D-glucose, α-methyl-D-glucoside, and D-galactose, but not 3-O-methyl-D-glucose, had a small inhibitory effect on myo-inositol reabsorption in short loops of Henle (Fig. 6). The effect of D-glucose was higher in the PCT (Fig. 8), where the low-affinity SGLT2 transporter is located. Small-to-moderate inhibition of myo-inositol uptake by D-glucose also has been shown in rat and rabbit BBMV (11, 27). Thus it is possible that myo-inositol is reabsorbed by the SGLT carriers. However, D-glucose uptake currents in oocytes transfected with canine SMIT are small and not different for D- and L-glucose. This observation hardly seems compatible with the hypothesis that SGLTs represent the apical myo-inositol transporters in the kidney because glucose transport by SGLTs is highly stereospecific.

To further test the hypothesis that myo-inositol is reabsorbed via one of the SGLT carriers, we characterized D-glucose reabsorption in the short loops of Henle (Fig. 7) and the PCT (Fig. 9) in the same way as we did myo-inositol reabsorption. In both segments, marked D-glucose reabsorption, which was phloridzin sensitive and saturable, took place. The roughly estimated kinetic constants were similar to those determined in earlier microperfusion experiments in vivo (2). However, a comparison with the kinetic constants for myo-inositol reabsorption also obtained in the present study shows that J and K for D-glucose reabsorption in the short loops of Henle are about fourfold higher than those for myo-inositol reabsorption.

D-Glucose reabsorption was inhibited to a moderate extent by α-methyl-d-glucoside in the short loop of Henle and by 3-O-methyl-D-glucose in the PCT. However, in the context of this paper, it is most important that neither myo-inositol nor D-chiro-inositol influenced D-glucose reabsorption in either segment to any extent. These results clearly show that the SGLT carriers do not accept myo-inositol to a significant extent, thereby confirming earlier results that showed that myo-inositol does not have any influence on radiolabeled α-methyl-D-glucoside uptake in SGLT1- (16) or SGLT2-transfected oocytes (14).

A further possibility is that tubular reabsorption of myo-inositol is mediated by the H+-myo-inositol symporter (HMIT) expressed predominantly in the mammalian brain (31) but apparently to a small extent also in the kidney. Increasing the driving force for H+ uptake into oocytes by decreasing the extracellular pH from 7 to 5 increased myo-inositol transport roughly sixfold (31). However, in the present work, increasing the pH of the microinfusate from 6.7 to 7.6 did not have any effect on myo-inositol reabsorption in the short loops of Henle. Thus it seems to be unlikely that the tubular reabsorption investigated in this paper is mediated by HMIT. The fact that the uptake of myo-inositol into tubular BBMV is driven by a Na+ gradient (10, 25) also speaks against this possibility and also against tubular reabsorption of myo-inositol by a uniporter like GLUT5.

Most recently, an orphan cDNA 43% identical in sequence to SMIT [now called SMIT1 (3)] was expressed in oocytes that were subsequently voltage clamped (3). Inward currents were found during superfusion with myo-inositol, D-chiro-inositol, and, to a smaller extent, with D-glucose. Uptake by this transporter (called SMIT2 by the authors) exhibited stereospecificity for D-glucose and D-chiro-inositol. L-Fucose was not accepted by SMIT2. This specificity resembles not only that found earlier when myo-inositol uptake was studied in liver cells (21) but also that of our present data. Thus we hypothesize that SMIT2 is responsible for renal tubular reabsorption of myo-inositol.

We conclude from our data that tubular reabsorption of myo-inositol in the PCT and in the loop of Henle is responsible for the nearly complete fractional reabsorption of this compound. Myo-inositol reabsorption is not mediated by the SGLTs, the HMIT carrier, the mannose transporter, or the GLUT5 uniporter. Our data support our hypothesis that myo-inositol reabsorption across the luminal membrane of the PCT, the short loop of Henle, and the ascending limb of the LLH is mediated by SMIT2. The extent to which this luminal route is used for the high myo-inositol accumulation in the cells of the thick ascending limb of Henle’s loop (23) and for renal intracellular inositol metabolism remains to be elucidated.

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REFERENCES


