Effect of α-ketoglutarate on organic anion transport in single rabbit renal proximal tubules

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Welborn, John R., Shlomo Shpun, William H. Dantzler, and Stephen H. Wright. Effect of α-ketoglutarate on organic anion transport in single rabbit renal proximal tubules. Am. J. Physiol. 274 (Renal Physiol. 43): F165–F174, 1998.—The effect of exogenous α-ketoglutarate (αKG) and the peritubular Na+-dicarboxylate (Na-DC) cotransporter on organic anion/dicarboxylate (OA/DC) exchange in S2 segments of single, nonperfused rabbit proximal tubules was measured using 1 µM fluorescein (FL), a model OA, and epifluorescence microscopy. The effect of different transmembrane distributions of 10 µM αKG on peritubular FL uptake was measured at 37°C using bicarbonate-buffered, nutrient-containing buffers, which are conditions similar to those found in vivo. Compared with FL uptake in the absence of exogenous αKG, preloading tubules with αKG (trans-configuration) or acute exposure to αKG (cis-configuration) increased FL uptake 62% and 54%, respectively, whereas a cis-trans-configuration of αKG increased FL uptake by 76%. The cis-stimulation of FL uptake by αKG was rapid, within 5–7 s. This stimulation was blocked 96% by simultaneous exposure to 2 mM Li⁺, indicating that stimulation of transport was secondary to the uptake of exogenous αKG. In the absence of exogenous αKG, selective inhibition of Na-DC cotransport using 2 mM Li⁺ or 1 mM methylsuccinate decreased FL uptake by 25% (effects that were reversible but not additive), suggesting that the Na-DC cotransporter recycles endogenous αKG that has left the cell in exchange for FL and that this activity supports ~25% of baseline activity of the OA/DC exchanger. With recycling of αKG accounting for ~25% of FL uptake and with accumulation of exogenous αKG accounting for another ~75% increase in FL uptake, Na-DC cotransport appears to directly support (25% + 75%)/175%, or ~57%, of total FL transport.

kidney; metabolic intermediates; fluorescein; cotransport; epifluorescence microscopy

THE KIDNEYS ELIMINATE a wide variety of organic anions (OAs) from the body, and mechanisms associated with the secretion of OAs by cells of the renal proximal tubule have received considerable attention (21). Transport of OAs into proximal cells from the blood, that is, transport across the basolateral (peritubular) membrane of proximal cells, is the active step in transmembrane secretion (21). Studies of Ullrich and colleagues (30, 32) on the selectivity of peritubular OA transport suggest that there is a single transport process, the “classic” OA transporter, that accepts a broad range of chemical structures and for which p-aminohippurate (PAH) has proven to be the prototypical substrate. Peritubular transport of PAH shows a wide degree of sensitivity to the presence or absence of sodium and potassium in the external medium, as well as to the presence of a wide range of metabolic intermediates, including many substrates of the tricarboxylic acid cycle (21).

In 1987 two research groups independently proposed a model that integrated effectively the broad range of observations on ionic and metabolite dependence of peritubular OA transport (18, 24). The model involves the concerted activity of three parallel transport processes in the peritubular membrane of renal proximal cells: the Na⁺-K⁺-ATPase; a Na⁺-dicarboxylate (Na-DC) cotransporter; and the classic OA transporter (referred to hereafter as the OA/DC exchanger), which mediates the exchange of extracellular OAs for a limited set of intracellular dicarboxylates. Active uptake of OAs is driven by an outwardly directed gradient of dicarboxylates [principally α-ketoglutarate (αKG)], which, in turn, is supported by the uptake of exogenous (extracellular) αKG by the Na-DC cotransporter. Steady-state activity of these functionally linked transport processes is ultimately dependent on Na⁺-K⁺-ATPase activity, which maintains the transmembrane electrochemical gradient for Na⁺. This “tertiary active” transport model has been demonstrated to operate in several different mammalian experimental systems, including isolated basolateral membrane vesicles (18, 19, 24), slices of intact renal cortex (20), and single isolated renal proximal tubules (3, 29). Functionally linked transporters for dicarboxylates and OAs have also been demonstrated in proximal tubules from reptilian (2) and teleost (13) kidneys and from the proximal-like epithelium of crustacean urinary bladder (15). Hence, the functional coupling of parallel transporters appears to be an ancient and highly conserved strategy to support active transport of OAs.

Two aspects of the tertiary active transport model remain untested. First, according to the model, OA uptake is increased as a consequence of transport of exogenous αKG. In fact, it is not known whether activity of the Na-DC cotransporter under physiological conditions increases uptake of OAs. Proximal tubule cells can actively accumulate PAH in the absence of exogenous dicarboxylates (e.g., Ref. 8), indicating that cellular metabolism can produce sufficient concentrations of “endogenous” αKG to support activity of the OA/DC exchanger. Most studies linking transport of exogenous dicarboxylates to the stimulation of OA transport have used concentrations of exchangeable dicarboxylates much higher than the ~10 µM concentration found in the plasma (17, 23). Although low concentrations of αKG have been shown to stimulate OA uptake into renal cells (17), the conditions under which these measurements were made differ markedly from conditions in vivo (in terms of temperature and buffer composition) and were likely to have reduced metabolic...
production of αKG. It is therefore premature to conclude that transport of exogenous αKG influences transport of OAs in vivo.

Second, the tertiary active transport model suggests that Na-DC cotransport serves to “recycle” the αKG that exchanges for extracellular OAs, thereby sparing the loss of this substrate and helping to maintain the intracellular αKG pool (18, 21, 22). Consistent with this notion, net loss of glutarate from proximal cells loaded with the radiolabeled compound is accelerated when the Na-DC cotransporter is inhibited with Li+ (20). However, a prediction that arises from the model is that the rate of OA transport should be sensitive to the activity of the Na-DC cotransporter, even in the absence of exogenous αKG. There is no such evidence.

We addressed these two issues in the present study by examining the effect of Na-DC cotransporter activity on the initial rate of OA transport. Epifluorescence microscopy and fluorescein (FL), a fluorescent OA, were used to measure activity of the peritubular OA transporter in single isolated rabbit proximal tubules (28). Our results suggest that, under conditions similar to those found in vivo, Na-DC cotransport plays a significant role in sustaining activity of the OA/DC exchanger, even in the absence of exogenous dicarboxylates.

METHODS

Chemicals

Spectral grade FL was purchased from Molecular Probes (Eugene, OR). Aminoxyacetate and N-methyl-D-glucamine (NMDG) were obtained from Sigma Chemical (St. Louis, MO). [3H]PAH was purchased from New England Nuclear (Boston, MA). Cell-Tak was obtained from Collaborative-Biomedical Products, Bedford, MA. All other chemicals were purchased from commercial sources and were of the highest available purity.

Solutions

A modified rabbit Ringer solution, used in the experiments as a dissection buffer, a superfusion buffer, and uptake medium, consisted of the following (in mM): 110 NaCl, 25 NaHCO3, 5 KCl, 2 Na2HPO4, 1.8 CaCl2, 1.8 MgSO4, 10 sodium acetate, 8.3 glucose, 5 alanine, 4 lactate, and 0.9 glycine; with the radiolabeled compound is accelerated when the Na-DC cotransporter is inhibited with Li+ (20). However, a prediction that arises from the model is that the rate of OA transport should be sensitive to the activity of the Na-DC cotransporter, even in the absence of exogenous αKG. There is no such evidence.

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Animals and Proximal Tubule Preparation

Adult male New Zealand White rabbits were purchased from Myrtle’s Rabbitry (Thompson Station, TN) and killed by intravenous injection with pentobarbital sodium. A kidney was immediately removed, perfused with a HEPES-sucrose buffer. The chamber floor consisted of a no. 1 glass coverslip coated with 1 µl of Cell-Tak. The chamber was transferred to the stage of an Olympus IMT microscope and superfused with buffer at 5 ml/min. The chamber was fitted with a water jacket, and its temperature, as well as that of the incoming superfusion buffers, was maintained at 37°C. By using two-way switching valves, superfusion buffers could be changed in a few seconds while maintaining a constant flow rate and temperature. A small vacuum line on the side of the chamber removed overflow. The inlet line was a needle mounted on an adjustable manipulator arm attached to the microscope stage so that it could direct the superfusion buffer toward a tubule placed anywhere on the coverslip.

Measuring FL Uptake Into Rabbit Proximal Tubules

Initial rates of FL uptake were calculated from measurements of epifluorescence intensity using the methods of Sullivan et al. (28) with minor modifications. A monochromator (Photon Technology International, Brunswick, NJ) equipped with a 75-W xenon lamp was used to generate excitation light at 490 nm (~1–2 nm). A 490-nm dichroic mirror (model 490DCLP; Omega Optical, Brattleboro, VT) directed excitation light to the proximal tubule segment through a ×40 oil-immersion fluor objective (1.3 NA, Nikon). Emitted light passed through a 520-nm long-pass filter (Omega Optical) before reaching a photomultiplier tube (model H1C120; Hamamatsu, Bridgewater, NJ). Photomultiplier output was recorded at 1-s intervals, using a multichannel scaling board and software (Oxford Instrument, Oak Ridge, TN) installed in a personal computer.

Figure 1A shows a fluorescence profile of FL exchange in the chamber, resulting from the switch from an FL-free buffer to one containing 1 µM FL. For this profile, the microscopic field did not include a tubule, and the microscope was focused at the same level as that used for the profile shown in Fig. 1B. Before adding FL, the signal was relatively low, 480 ± 30 photons/s, representing instrument background. Background fluorescence increased rapidly to 364,000 ± 960 (SD) photons/s when FL was added to the bath, yielding a signal-to-noise ratio of ~750. This “solution background” was very stable: there was no significant change in slope during the period measured, and the standard deviation of the signal was less than 0.3% of the average. The fluorescence measured was a function of both monochromator slit width and microscopic field size, which varied between experiments but not within an experiment. As a result, the signal-to-noise ratio remained constant between experiments.

Figure 1B presents a fluorescence profile to show how initial rates of FL uptake were calculated. For this profile, the microscope was focused on a tubule (midpoint of tubule depth) in the chamber. Tubule autofluorescence at FL wavelengths was 2,580 ± 49 (SD) photons/s, a 5.4-fold increase in signal over instrument background. Upon switching to a superfusion buffer containing 1 µM FL, the increase in signal had two evident phases: a rapid increase in fluorescence due to the addition of FL to the bath and a slower increase in fluorescence indicating accumulation of FL in the tubule. Because the half time (t1/2) for solution exchange in the chamber was 1.5 to 2 s, the first 5–10 s of the fluorescence record (i.e., ~5 half times) was discarded following the switch to a buffer containing FL. The next 25 s of the record was linear, as shown by the t = 10–35 s portion of the trace in Fig. 1B. The slope of this line was calculated and represents the initial rate of FL uptake. For example, using this standard approach, the slope calculated for Fig. 1B was 4,790 photons/s, 

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The influences of PAH, Li\(^+\), or methylsuccinate on the initial rate of FL uptake were tested using the superfusion buffer containing 1 µM FL plus the test substrate in the cis-configuration. The effect of sodium’s absence on FL transport was determined by replacing sodium in the uptake medium with NMDG. The initial rates of FL uptake were first measured under the control condition (both superfusion buffer and uptake media contained ~150 mM sodium). Tubules were then superfused for 10 min with superfusion buffer, and FL uptake rates were measured using uptake medium containing FL and NMDG. When measuring the effects on FL transport resulting from various transmembrane configurations of αKG or other experimental treatments (e.g., cis-inhibition), two to four measurements of FL uptake in the control condition and two to four measurements of FL uptake in the experimental condition were made per tubule.

Measuring Uptake of PAH Into Rabbit Proximal Tubules

The peritubular uptake of [\(^3\)H]PAH into isolated rabbit proximal tubules was measured using the methods of Shpun et al. (25).

Statistical Analysis

Unless indicated otherwise, data are means ± SE. Sample size (n, m) refers to n separate tubules from m different rabbits. Comparisons of observed differences to determine their statistical significance at the 0.05 level, were performed using either a one-way, two-sample t-test or an analysis of variance (ANOVA) and a post-test employing the Student-Newman-Keuls method. The statistical tests performed on each data set are indicated in the legends to Figs. 1–7.

RESULTS

Time Course of FL Accumulation and Precision of Initial Rate Measurements

Figure 2A shows a time course of FL uptake into and efflux from a proximal S2 tubule segment. Accumulation of FL into the tubule segment, started by switching the superfusion buffer to a buffer containing 1 µM FL, approached steady state within ~10 min. Switching the superfusion buffer to a buffer containing no FL initiated efflux of FL from the tubule. The background level of tubule autofluorescence was reached after ~20 min of efflux.

When the initial rate of FL uptake was repeatedly measured in a single tubule, exposure to the uptake medium was limited to 30–35 s. Accumulated FL was then washed out of the tubule by superfusing the tubule for 10 min using an FL-free buffer. Figure 2B shows 4 representative measurements of uptake from an experiment where 15 replicates of FL uptake into a tubule were measured sequentially over a 3-h period using 20 µM αKG in the cis-trans configuration and 1 µM FL. Typically, FL uptake rates were very reproducible when measured using this brief-exposure protocol and seldom declined by more than 15% over a 4-h
period. If uptake rates in controls declined by more than 15%, then a mean uptake rate was calculated from rates measured in controls before and after the experimental treatment. The mean uptake rate was then compared with the rates of FL uptake measured under the experimental condition.

Interaction of FL with PAH Transport

The use of FL as a model substrate for the peritubular OA/DC exchanger assumes that FL uptake is effectively limited to an interaction with that transporter. PAH has long been used as a model compound for studying the characteristics of the OA/DC exchanger, and there is strong evidence indicating that entrance of PAH into proximal tubule cells across the peritubular membrane is limited to this single process (30). Therefore, we sought to establish that FL transport involves and is limited to the "PAH transporter." Interaction with the PAH transporter was supported by data presented in Fig. 3A, showing that the initial rate of [3H]PAH uptake into single rabbit proximal S2 segments was inversely related to the concentration of FL in the uptake medium. The kinetic profile suggested a competitive interaction with an apparent inhibitory constant (K_i) of 15 ± 3.8 µM (n = 3; m = 3). The similarity between this K_i value and the half-saturation constant (K_M) of 10 µM for peritubular FL uptake into single rabbit proximal tubules (28) supports the idea that FL shares the OA/DC exchanger with PAH.

The second criterion, i.e., that transport of FL should be limited to the PAH transporter, was supported by the observation that 5 mM PAH in the uptake medium reduced the initial rate of FL uptake into single tubules by 95% (Fig. 3B), suggesting that peritubular transport of FL is effectively limited to the OA/DC exchanger. These results agree with the report that PAH inhibits FL transport into rabbit proximal S2 segments with a K_i of 141 µM, similar to the 110 µM value of K_M for peritubular PAH transport (5). Taken together, these results allow us to conclude that FL is comparable to PAH as a model substrate for the peritubular OA/DC exchanger.
Influence of "Physiological" Concentrations of Exogenous αKG on Peritubular FL Transport

Most studies to date have used 100 µM αKG (or glutarate) in the trans-configuration to study the influence of dicarboxylates on OA transport in mammalian renal tubules (2, 3, 29), i.e., isolated tubules have been preloaded with this relatively large concentration of dicarboxylate, followed by a measurement of OA uptake using a dicarboxylate-free buffer. This protocol has served to emphasize the stimulatory effect of dicarboxylate uptake on OA transport by maximizing the transmembrane gradient of dicarboxylates and by eliminating the cis-inhibition caused by a competitive interaction between dicarboxylates and OAs at the extracellular face of the OA/DC exchanger. However, the concentration of αKG in the blood is only 10 µM (23). Therefore, we measured the trans-stimulation of FL transport resulting from exposure to 10 µM αKG and compared it to the trans-stimulation resulting from exposure to 100 µM αKG. As shown in Fig. 4A, although 100 µM αKG trans-stimulated FL uptake by 81 ± 15% (n = 9, m = 8), 10 µM αKG-stimulated FL transport, on average, to the same degree, i.e., 62 ± 27% (n = 8, m = 8).

Unlike the situation produced by the trans-configuration, transport of OAs in vivo occurs under conditions where exchangeable dicarboxylates are distributed at steady state across the peritubular membrane, i.e., a cis-trans configuration. At least one study has examined the effect of a steady-state "cis-trans" distribution of a low concentration of αKG on the activity of the OA/DC exchanger. Pritchard (17) showed that simultaneous exposure of rat renal cortical slices to a solution containing 10 µM [3H]PAH and 10 µM αKG causes a 30% increase in the steady-state tissue/medium ratio of PAH accumulation. Although these data suggest that a concentration and transmembrane configuration of αKG found in vivo can stimulate accumulation of OAs, this interpretation is complicated by the fact that the tissue was incubated at room temperature in a nutrient-free, nominally bicarbonate-free, phosphate buffer. These conditions are likely to have influenced cellular metabolism and/or rates of OA transport. Thus we sought to clarify the extent to which exogenous αKG influences peritubular OA transport under conditions similar to those in which proximal tubules are normally exposed in vivo.

We compared the effect of the three different αKG exposure protocols, the cis-, trans-, and cis-trans-configurations, on the uptake of 1 µM FL at 37°C using an extracellular concentration of 10 µM αKG and a bicarbonate buffer. Two observations were of particular interest. First, as shown in Fig. 4B, all three αKG configurations significantly stimulated, by 54% to 76%, uptake of 1 µM FL, compared with the control. These data support the conclusion that exposure of tubules to exogenous αKG under conditions that mimic those found in vivo (with respect to temperature, substrate concentration, and buffer composition) can significantly increase the rate of peritubular OA transport. Because of the variability in the degree of stimulation produced by tubules from different rabbits, a simple one-way repeated measures ANOVA did not indicate significant differences between the different transmembrane configurations of αKG. However, we think it is also worth noting that of the five trans and cis-trans pairings, in four cases FL uptake measured under the cis-trans-configuration exceeded that measured under the trans-configuration (in the 5th case, the rates were virtually identical). This observation suggests that the continuous accumulation of exogenous αKG produces a
higher concentration of this exchangeable substrate at the cytoplasmic face of the OA/DC exchanger than can be sustained by diffusion from the elevated cytoplasmic pool of αKG.

The second point apparent from the experiments summarized in Fig. 4B was that the increase in FL transport resulting from exposure to exogenous αKG can occur quite rapidly. Under the cis-configuration, the 22–160% increase in FL transport we observed in 11 separate experiments occurred within the 5–7 s that represented the limit of temporal resolution of our present technique. The rapid onset of the cis-stimulation is evident in the data presented in Fig. 5. In this experiment, acute exposure of a tubule to 10 µM αKG immediately increased the initial rate of FL uptake by 85% compared with the control condition. Simultaneous exposure of the tubule to 10 µM αKG and 2 mM Li⁺ essentially eliminated the αKG-mediated stimulation of FL transport. In five measurements from two separate tubules, 2 mM Li⁺ blocked 96% of the increase in FL uptake produced by 10 µM αKG under the cis-configuration. Because Li⁺ is a specific inhibitor of the Na-DC cotransporter (1, 19, 31), we concluded that the increased transport produced by the cis-configuration was secondary to the uptake of exogenous αKG. The increase in FL uptake presumably reflected an increased turnover of the OA/DC exchanger that was concomitant to an increase in the intracellular αKG concentration, an increase that must have been at least proportional to the increase in FL transport. In other words, the stimulation of FL transport noted under the cis-configuration must have been secondary to an increase of 22% to 160% in the concentration of αKG at the cytoplasmic face of the exchanger. The rapidity of the response necessitates that the increase in αKG occur within a few seconds, a subject we address in the DISCUSSION.

Effect of DC Transport on FL Transport in Absence of Exogenous αKG

A central element of the tertiary active transport model for peritubular OA transport is the recycling of αKG lost from proximal cells after exchange for extracellular OAs (18, 21, 22). It is not clear, however, the extent to which this recycling occurs in vivo or its potential influence on the rate of peritubular OA transport. To determine the direct influence of Na-DC cotransport on the rate of FL uptake, activity of the Na-DC cotransporter was eliminated by removing Na⁺ from the uptake medium. These studies were performed in the absence of exogenous αKG in the medium. When tubules were acutely exposed to superfusion buffer in which Na⁺ was replaced with NMDG (Fig. 6A), the initial rate of uptake of 1 µM FL was immediately reduced by 81.6 ± 1.3% (P < 0.05; n = 5; m = 4), an inhibition that was fully reversible.

Although these data are consistent with the idea that activity of the OA/DC exchanger is functionally linked to activity of the Na-DC cotransporter, it is difficult to dismiss the possibility that removal of Na⁺, even transiently, could have influenced cellular processes that exert indirect effects on peritubular OA transport. Consequently, we examined the effects of two comparatively selective inhibitors of the Na-DC cotransporter on the rate of FL transport. The first was exposure to 1 mM methylsuccinate, and the second was exposure to 2 mM Li⁺. Methylsuccinate is a high-affinity substrate (Kᵣ = 10 µM) of the peritubular Na-DC cotransporter in rabbits (1) and a relatively weak inhibitor of the OA/DC exchanger (33). Thus a sufficiently large concentration of methylsuccinate in the uptake medium should prevent αKG from entering proximal cells by the Na-DC cotransporter. A concentration of 1 mM methylsuccinate was selected, because in rabbit proximal tubules it reduces by more than 95% the stimulatory effect of exogenous αKG on PAH uptake (3). As noted previously, Li⁺ is a relatively specific inhibitor of the peritubular Na-DC cotransporter (1, 19) that competes with Na⁺ for binding to the cotransporter and produces an activator-transporter complex with an extremely low affinity for dicarboxylates. Chatsudhipong and Dantzer (3) showed that 2 mM Li⁺ eliminates the stimulation of PAH transport into rabbit renal proximal tubules produced by exposure to 100 µM αKG. The results presented in Fig. 5 confirmed that 2 mM Li⁺ blocks the accumulation of exogenous αKG.

We tested the effects of Li⁺ and methylsuccinate on FL transport in separate experiments with tubules superfused with buffer containing no exogenous αKG. Acute application of 2 mM Li⁺ or 1 mM methylsucci-
Fig. 6. A: effect of sodium removal on the uptake of 1 μM FL into single renal proximal S2 tubule segments. Sodium in the uptake medium was replaced with N-methyl-D-glucamine (NMDG), whereas the control condition contained the normal concentration of sodium. Height of each bar is mean ± SE of uptake measured in 5 tubules (m = 4). Buffers used for these experiments did not contain αKG. *Decrease in initial rate of FL uptake was significant (P < 0.05) compared with controls, as determined by using a one-way, two-sample t-test. B: effect of 2 mM Li+ and 1 mM methylsuccinate (MS) on 1 μM FL uptake into a single renal proximal S2 tubule segment. Height of each bar represents uptake as mean ± SE of 2–3 sequential measurements of FL uptake in presence of one or both of the test agents. Control bar is mean ± SE of 9 measurements of initial rate of uptake measured before and after each of the test conditions. All buffers used for this experiment did not contain αKG. *Decrease in initial rate of FL uptake was significant (P < 0.05) compared with controls, as determined by ANOVA.

nate significantly (P < 0.05) reduced FL uptake as follows: Li+ by 18 ± 5.0% (n = 3, m = 3) and methylsuccinate by 29 ± 2.3% (n = 3, m = 3). Significantly, in neither case was the degree of inhibition of FL transport as substantial as that produced by complete removal of Na+ from the medium (Fig. 6A). If the effects of Li+ and methylsuccinate are through a common mechanism, i.e., inhibition of the Na-DC cotransporter, then their effects should not be additive. This hypothesis was tested in a separate experiment that used a single tubule, the results from which are summarized in Fig. 6B. Consistent with the observations reported above, Li+ and methylsuccinate each reduced the rates of FL uptake into the tubule by ~28%, inhibitions that were completely reversed by reexposing the tubule to control buffer. When the tubule was then simultaneously exposed to 2 mM Li+ plus 1 mM methylsuccinate, the inhibition of FL uptake (33%) was not different from that produced by either Li+ or methylsuccinate alone (P > 0.05). Thus the effects of Li+ and methylsuccinate appear to be limited to inhibition of the Na-DC cotransporter. The consistent observation that selective inhibition of the Na-DC cotransporter in the absence of exogenous αKG simultaneously reduced FL transport by ~25% suggests that 25% of peritubular OA/DC exchanger activity requires parallel activity of the Na-DC cotransporter.

DISCUSSION

The current observations support two conclusions. First, under conditions that mimic closely those found in vivo (i.e., substrate concentration, buffer composition, incubation temperature), exogenous αKG (that is, αKG in the extracellular medium, in contrast to endogenous, metabolically produced αKG) significantly stimulates the peritubular uptake of FL, a model OA. Second, activity of the peritubular Na-DC cotransporter significantly enhances OA uptake, even in the absence of exogenous αKG.

Prior to these observations, firm conclusions about these issues were not possible. Although it was apparent that peritubular OA transport involves mediated exchange with intracellular αKG (18, 24) and that uptake of exogenous αKG stimulated OA uptake, it was also evident that isolated renal tissue can actively accumulate OAs in dicarboxylate-free medium (e.g., Ref. 8). Thus it was not known whether uptake of exogenous αKG would increase cytoplasmic αKG to levels above that already present due to cellular metabolism. Also, previous studies frequently used nonbicarbonate buffers (14, 17, 20), nutrient-free media (11, 14, 17, 20), and nonphysiological incubation temperatures (17, 20). Any of these conditions is likely to have affected the rates of transport and/or cellular metabolism in ways that altered the influence that DC transport had on the transport of OAs. For example, in rabbit renal proximal cells, exposure to bicarbonate-free buffers decreases the pool size of cytosolic dicarboxylates in parallel with increases in the mitochondrial pool size of dicarboxylates (26). In intact renal proximal tubules from dog, low-bicarbonate conditions decrease cytosolic concentrations of αKG without changing the concentration of mitochondrial αKG (27). Bicarbonate deletion also results in a direct inhibition of mitochondrial respiration and a decrease in the cytosolic pool sizes of tricarboxylic acid cycle intermediates in rabbit proximal tubules (6). These observations all suggest that rates of OA transport measured in the absence of bicarbonate will reflect the influence of low cytoplasmic concentrations of αKG. In that regard, it is relevant to
note that exposure of intact rabbit proximal tubules to nominally bicarbonate-free (HEPES-containing) buffers reduces the rate of peritubular transport of PAH (3, 4) and FL (Welborn, unpublished results). Thus, despite previous observations showing that micromolar concentrations of αKG can stimulate renal OA transport (17), it was difficult to assess the extent to which DC transport stimulates OA transport under physiological conditions.

The present observations were made using concentrations of αKG that proximal tubules are exposed to in vivo (23). Exposing tubules to 10 µM αKG significantly stimulated FL uptake under all conditions tested (Figs. 4 and 5). The condition most representative of the in vivo condition, i.e., cis-trans exposure of tubules to 10 µM αKG in a bicarbonate buffer, increased the initial rate of FL uptake by 76% over that measured in the absence of exogenous αKG (Fig. 4), indicating that uptake of exogenous αKG can support 43% of total uptake of FL (i.e., 76%/176%).

The second conclusion supported by this study is that activity of the peritubular Na-DC cotransporter significantly enhances the renal uptake of OAs, even in the absence of exogenous αKG. The tertiary active transport model for renal OA transport includes the recycling of αKG across the membrane as the Na-DC cotransporter and the OA/DC exchanger operate in parallel (18, 22). However, there has been no evidence indicating the extent to which this functional interaction occurs under physiological conditions. Removal of Na+ from the external medium, which would eliminate activity of the Na-DC cotransporter, almost completely eliminated FL uptake (Fig. 6A), consistent with the effect of Na+ removal on OA transport reported in previous studies (12, 34). However, it is not apparent the degree to which this inhibition was influenced by the effect of Na+ removal on other cellular processes. The 25% inhibition of FL transport produced by a selective inhibition of the Na-DC cotransporter, although more modest than that produced by Na+ removal, is probably a more accurate indicator of the extent to which activity of Na-DC cotransporter results in the reaccumulation of αKG lost from proximal cells through the OA/DC exchanger. This result also supports the contention that recycling of endogenous αKG can support 25% of the peritubular transport of OAs.

The present measurements permit an estimate of the influence that Na-DC cotransporter has on the rate of peritubular OA/DC exchange. Recycling of αKG across the peritubular membrane supports ~25% of the “basal” activity of the OA/DC exchanger (Fig. 6B). i.e., the activity that is supported by cellular production of αKG. Also, the peritubular membrane at steady state in vivo is exposed to 10 µM αKG in the cis-trans configuration, and under this configuration, αKG increased the rate of FL transport by ~75% over the basal rate (Fig. 4B), presumably by increasing the cytoplasmic concentration of αKG. These two observations suggest that Na-DC cotransporter activity is directly responsible for supporting ~57% [(75%+25%/175%) of the total peritubular flux of OAs via the OA/DC exchanger. Figure 7 presents a model emphasizing the two modes of influence of the Na-DC cotransporter on the activity of the OA/DC exchanger.

We have assumed that the stimulation of FL transport produced by exposing tubules to exogenous αKG was the result of an increase in the intracellular concentration of αKG and its effect on turnover of the OA/DC exchanger. This assumption is supported by many observations including the mediated exchange of PAH for αKG and structurally related dicarboxylates in isolated basolateral membrane vesicles (19, 24) and the direct effect that intracellular αKG concentration has on the rate of peritubular PAH transport in renal cortical slices (17). It is also unlikely that a stimulation of cell metabolism and consequent changes in cell ATP content was responsible for the increase in FL transport caused by exposure to exogenous αKG. First, exposing tubules to 100 µM αKG under conditions similar to those used in the present study has no effect on cell ATP content (2). Moreover, active uptake of OAs has been shown to occur under conditions that should have drastically curtailed cellular metabolism, suggesting that cell ATP content has no direct effect on peritubular OA transport (16). Therefore, an increase in OA uptake following exposure to αKG is probably secondary to an increase in the concentration of αKG at the cytoplasmic face of the OA/DC exchanger. As noted earlier, the rapidity with which exposure to αKG stimulates FL transport (e.g., Fig. 5) necessitates that equally rapid and proportionately large increases occur in the cytoplasmic concentration of αKG.

It appears relevant to discuss the implications of such changes in the light of what is known about the cytoplasmic pool of αKG and rates of OA transport. The Fig. 7. Model of the organization of peritubular transporters involved in the “tertiary active” transport of FL into renal proximal tubule cells. Transporter 1 is the organic anion/dicarboxylate (OA/DC) exchanger, which accumulates extracellular FL through mediated exchange with intracellular αKG. Active uptake of FL is supported by an outwardly directed αKG gradient maintained by cellular metabolism and activity of the Na-DC cotransporter. Transporter 2 is the Na-DC cotransporter, which, through accumulation of exogenous αKG and recycling of endogenous αKG, elevates the concentration of αKG at the cytoplasmic face of the OA/DC exchanger. Transporter 3 is the Na+-K+ ATPase, which maintains the inwardly directed Na+ electrochemical gradient that supports activity of the Na-DC cotransporter.
cytoplasmic αKG concentration in renal proximal cells is ~200 µM (17), which is near the apparent \( K_t \) for αKG interaction with the OA/DC exchanger (17). Consequently, a 54% increase in the activity of the OA/DC exchanger resulting from exposure to 10 µM αKG in the cis configuration (Fig. 4B) would require, at least, a 54% increase in the concentration of αKG at the cytoplasmic face of the exchanger; i.e., an increase of 108 µmol/liter cell water. Although the kinetics of peritubular αKG uptake have, to our knowledge, not been measured, Ullrich and colleagues (7) have measured the maximal rate of Na\(^+\)-methylsuccinate cotransport in intact rat proximal tubules (7): 0.5 pmol·cm\(^{-1}\)·s\(^{-1}\) or ~1,400 µmol·liter cell water\(^{-1}\)·min\(^{-1}\). The \( K_t \) of αKG’s interaction with this process is 80 µM (31), from which we can infer an uptake rate of ~17.5 µmol/liter cell water from an external αKG concentration of 10 µM during the 5–7 s required to resolve the cis-stimulation of FL transport (Fig. 5). While acknowledging that the rate of peritubular αKG transport may be substantially greater in rabbit proximal tubules, it still seems unlikely that the accumulation of αKG would be sufficiently rapid to increase the cytoplasmic concentration by more than 100 µM within a few seconds.

Alternatively, uptake of exogenous αKG could elevate the local αKG concentration at the peritubular face of the OA/DC exchanger. Consistent with this idea is the observation that rates of FL transport were typically higher when measured under the cis-trans configuration than under the trans-configuration (Fig. 4B), despite the fact that both configurations should have included the same intracellular concentration of αKG. In other words, it appears that continuous uptake of exogenous αKG supported a higher rate of transport than that arising from simply loading the entire cell with αKG. This observation could be explained if Na-DC cotransport increased the local concentration of αKG near the cytoplasmic face of the OA/DC exchanger. It would, however, be premature to conclude that the Na-DC cotransporter could support a standing gradient of αKG concentration within the cell. Similar ideas have been suggested and rejected with respect to the potential influence of transport processes within the microvillus brush border of some epithelial cells (9). Diffusion is quite fast over distances of a few microns, making it difficult for a gradient to be sustained between two points within a cell. For membrane transport to be effective in producing a local concentration substantially larger than that in the bulk cytoplasm, some rather severe constraints must be imposed on the system involving both the rate of transport (it must be large) and the morphology of the space into which solute is transported (it must be restricted). The complex morphology of the basolateral aspect of proximal tubule cells (35) offers some interesting possibilities with respect to this idea, but much more information on transport rates and cellular morphometry will be required before conclusions can be drawn about the subcellular mechanisms of solute coupling between the peritubular transport of αKG and OAs.

In conclusion, under physiological conditions, accumulation of exogenous αKG into renal proximal tubules occurs at rates sufficient to significantly increase the peritubular uptake of the organic anion FL, probably due to an increase in the intracellular αKG concentration. Activity of the Na-DC cotransporter also supports the peritubular OA/DC exchange by recycling cellular αKG. As a result of these separate influences on activity of the OA/DC exchanger, we conclude that the Na-DC cotransporter directly supports ~60% of the renal transport of OAs.

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