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Review

Renal organic anion transport: a comparative and cellular perspective

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Abstract

A major system for net transepithelial secretion of a wide range of hydrophobic organic anions (OAs) exists in the proximal renal tubules of almost all vertebrates. This process involves transport into the cells against an electrochemical gradient at the basolateral membrane and movement from the cells into the lumen down an electrochemical gradient. Transport into the cells at the basolateral membrane, which is the dominant, rate-limiting step, is a tertiary active transport process, the final step which involves countertransport of the OA into the cells against its electrochemical gradient in exchange for α-ketoglutarate moving out of the cells down its electrochemical gradient. The outwardly directed gradient for α-ketoglutarate is maintained by metabolism (~ 40%) and by transport into the cells across both the basolateral and luminal membranes by separate sodium-dicarboxylate cotransporters (~ 60%). The inwardly directed sodium gradient driving α-ketoglutarate uptake is maintained by the basolateral Na^+–K^+–ATPase, the primary energy-requiring transport step in the total tertiary process. The basolateral OA/α-ketoglutarate exchange process now appears to be physiologically regulated by several factors in mammalian tubules, including peptide hormones (e.g., bradykinin) and the autonomic nervous system acting via protein kinase C (PKC) pathways and epidermal growth factor (EGF) working via the mitogen-activated protein kinase (MAPK) pathway.

Keywords: Renal tubule; Organic anion; Basolateral transport; Luminal transport; Coupled transport

1. Introduction

A major system for the net secretion of a wide range of hydrophobic organic anions (or weak organic acids that exist as anions at physiological pH; collectively OAs) apparently exists in the renal tubules of all vertebrates studied except a species of urodele amphibian (*Necturus maculosus*) [1,2] and a species of hagfish (*Myxine glutinosa*) [3,4]. The renal tubules of *M. glutinosa* apparently do not transport OAs [3,4] and those of *N. maculosus* show net reabsorption of some OAs (although net secretion is occasionally observed) or no net transport of other OAs [1,2].

The general OA secretory system found in most vertebrates involves transport from the blood (or peritubular) side of the renal tubule across the epithelium into the tubule lumen. Indeed, it was this system that E.K. Marshall used in the late 1920s to demonstrate in the aglomerular goosefish (*Lophius piscatorius*) that renal tubules could secrete substances (i.e., transport them from blood to tubule fluid) [5]. In these animals, OA could not be contributed to the urine by filtration. Some of the substances secreted by this system, e.g., organic dyes, could be measured easily by the techniques available in the 1920s and 1930s. As techniques became available to study the transport of inorganic ions, such as Na^+ and K^+, interest in OA secretion declined until quite recently. This is unfortunate because the OA transport system, although capable of secreting a number of endogenous compounds, is particularly effective in secreting numerous exogenous compounds, including many pharmacologically active substances, environmental toxins, and plant and animal toxins. It also secretes toxic metabolic breakdown products of exogenous and endogenous compounds. Indeed, its primary function appears to be the effective removal of such substances from the body. Although there are a number of other systems for transporting specific types of OAs in either the secretory or reabsorptive directions, I will not discuss these in any detail here. I shall concentrate on only a few aspects of the general system for the net secretion of OAs, with *para*-amino-

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Table 1
Tubule site of transport of organic anions

Species	Tubule site	References
Fishes (flounder)	Proximal tubule (all portions)	[6]
Amphibians (frog)	Proximal tubule (early and intermediate portions)	[8]
Reptiles (snake)	Proximal tubule (distal portion)	[7]
Birds (chicken)	Proximal tubule (all portions)	[31]
Mammals (rabbit)	Proximal tubule (S2 portion)	[73]

hippurate (PAH) and fluorescein (FL) as the prototypical transported anions.

2. Transepithelial transport

The technique of perfusing segments of renal tubules teased from fresh tissue without the aid of enzymatic agents has been used by many laboratories, including ours, to demonstrate initially or to confirm many aspects of the net secretory process for OAs. As indicated in Table 1, the proximal tubule has been shown by this technique to be the site of net secretion in representative species from all five vertebrate classes. However, the portion of the proximal tubule that is most important for net secretion varies among species (Table 1).

Saturation of the net transepithelial transport system has been demonstrated by both the clearance technique and the isolated perfused tubule technique in species across the vertebrate classes, and K_t and J_{max} values for the transport of prototypical OAs have been determined for a number of mammalian and nonmammalian species [6–10]. The kinetic values for mammalian (rabbit) tubules are about the same whether one looks at transepithelial transport or at transport into the cells at the basolateral side only, thereby indicating that the basolateral entry step is the rate-limiting step [9,11].

During the net transepithelial secretory process in isolated perfused mammalian and nonmammalian renal proximal tubules, the steady-state concentration of OA in the tubule lumen is greater than that in the peritubular bathing medium and the concentration in the cells is greater than that in either the lumen or the peritubular bathing medium [12,13]. Because the inside of the cells is negative compared with the lumen and the peritubular fluid, because these compounds are transported as anions at physiological pH, and because they do not appear to be bound inside the cells, these data are compatible with transport into the cells against an electrochemical gradient at the basolateral mem-

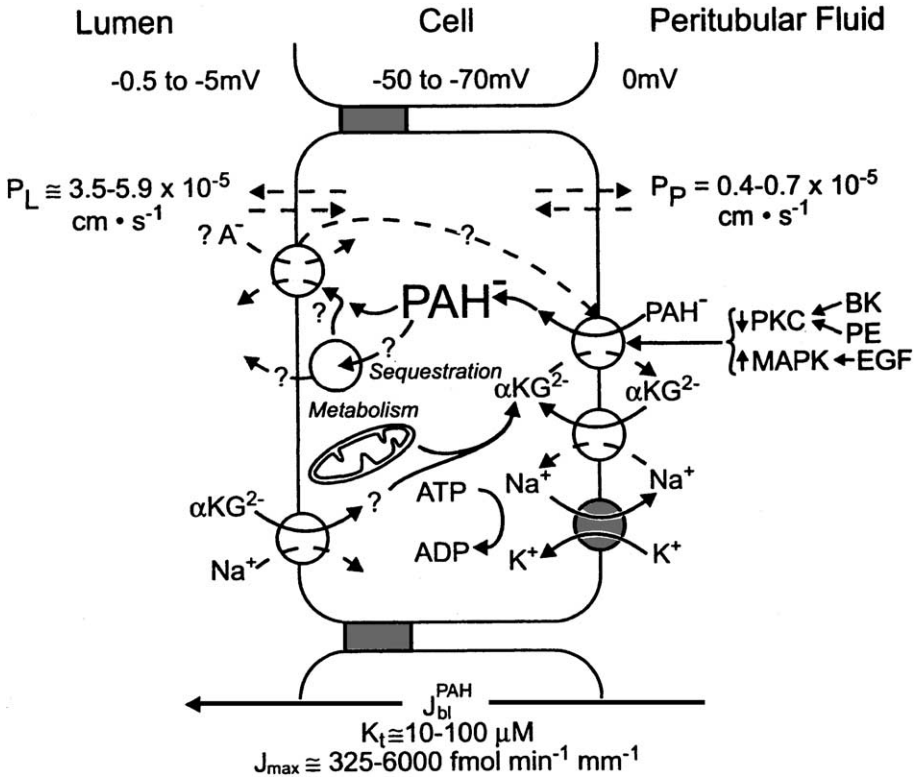

Fig. 1. Descriptive model of the steps in transepithelial transport of organic anions in proximal renal tubules, with PAH as a model substrate. This is based on work on mammals, birds, reptiles, amphibians, and fishes. Values for luminal (P_L) and peritubular (P_L) membrane permeabilities are from those given in Table 2. Values for kinetic constants are from Refs. [6–10]. Broken arrows with open circles indicate movement down an electrochemical gradient. Solid arrows with open circles indicate movement against an electrochemical gradient by some form of secondary active transport. Solid arrows and solid circle indicate primary active transport. A^- indicates anion of unknown type; αKG^{2-} represents α-ketoglutarate. Broken arrow with question mark indicates some form of possible feedback from transport step across luminal membrane to transport step across basolateral membrane.

Table 2
Apparent permeabilities of tubule cell membranes to PAH

Species	Luminal membrane ($cm\ s^{-1} \times 10^{-5}$)	Peritubular membrane ($cm\ s^{-1} \times 10^{-5}$)	References
Frog	3.8	0.7	[8]
Snake	3.5	0.5	[7,12]
Rabbit	5.9	0.4	[13]

brane and movement from the cells into the lumen down an electrochemical gradient. This process appears to be essentially the same for perfused flounder, frog, snake, chicken, and rabbit tubules and, indeed, is the accepted process for transepithelial secretion (Fig. 1).

The apparent permeabilities of the luminal and basolateral membranes to PAH have been measured from the fluxes across these membranes in isolated tubules from frogs, snakes, and rabbits (Table 2) [7,8,12,13]. The apparent permeability of the luminal membrane is always substantially greater than that of the basolateral membrane. This is appropriate and in agreement with the general model if PAH transported into the cells at the basolateral membrane is to move into the lumen and not back into the bath. For tubules from most species, the backflux of PAH from lumen to peritubular bathing medium is small and, for the species shown in Table 2, can be predicted almost perfectly from these independently measured luminal and basolateral membrane permeabilities, thus indicating that this small backflux occurs across the cells [12–14].

3. Basolateral transport

Although it could easily be shown that transport into the tubule cells across the basolateral membrane required energy [15,16], it could never be shown that it was a primary active transport process, i.e., that it was directly linked to the hydrolysis of ATP [17,18]. Moreover, although the process was shown to be Na^+-dependent, it could not be shown that it was directly linked to the Na^+-gradient [19–23]. However, evidence for (1) basolateral PAH/PAH exchange [19,21,22]; (2) inhibition of basolateral PAH uptake by the anion exchange inhibitor SITS [24]; (3) inhibition or stimulation of basolateral PAH uptake by a number of anionic metabolites, whose entry across the basolateral membrane was directly coupled to the Na^+-gradient; and (4) production of a brief basolateral uptake of PAH above equilibrium by a combination of an outwardly directed PAH or OH^- gradient and an inwardly directed Na^+-gradient [21,25] suggested to a number of investigators that PAH entry across the basolateral membrane might involve exchange for an anionic metabolite [19,23,26,27]. In 1987 and 1988, Burckhardt and Pritchard and their co-workers, using rat basolateral membrane vesicles (BLMV), extended this concept to develop the model shown in Fig. 1 [28–30]. In this model, transport into the cells of PAH (or other OAs that share this system) at the basolateral membrane is a tertiary active transport process, the final step in which is the countertransport of PAH against its electrochemical gradient in exchange for an intracellular dicarboxylate [physiologically, α-ketoglutarate (αKG)] moving down its electrochemical gradient. The outwardly directed gradient for αKG is maintained in turn by metabolism and by transport into the cells across the basolateral membrane by the Na^+-dicarboxylate co-transport system (Fig. 1). The inwardly directed Na^+-gradient driving this process is maintained by the primary energy-requiring transport step in the process, the transport of Na^+ out of the cells by Na^+–K^+–ATPase at the basolateral membrane.

As noted above, this model was initially developed in studies using rat basolateral membrane vesicles. We have demonstrated that it apparently does hold for OA transport in intact single perfused snake, chicken, and rabbit tubules [11,31–33]. When tubules are preloaded with αKG from the bathing medium, both uptake of PAH into the cells and net transepithelial secretion increase to a comparable extent as illustrated for snake renal tubules in Fig. 2. This stimulatory effect is eliminated if the preloading of the tubules with αKG via the Na^+-dicarboxylate cotransporter is prevented by inhibiting this transporter with Li^+ [31–33]. Analysis of the kinetics of PAH uptake at the basolateral membrane of isolated rabbit tubules clearly showed that the increase in PAH uptake with αKG preloading results from an increase in J_{max} with little change in K_t [11]. We also demonstrated the basolateral countertransport of PAH for αKG by measuring the stimulatory effect of αKG in the bathing medium on the rate of efflux of PAH across the basolateral membrane of tubules already loaded with PAH (i.e., the reverse effect) when the Na^+-dicarboxylate cotransporter was inhibited [32,33].

The metabolic state of the tubules, and thus their production of intracellular αKG, appears to be important (see below) especially for mammalian renal tubules. For example, in the case of rabbit tubules, the control level of PAH secretion is about five times as high when the tubules are perfused and bathed in a bicarbonate/CO_2-buffered medium as when they are perfused and bathed with any other buffer system (e.g., HEPES, Tris, phosphate) ([11]; S Shpun and W.H. Dantzler, unpublished observations). Moreover, apparently because of this high control rate of PAH secretion, transport is stimulated only about two-fold by αKG preloading in a bicarbonate/CO_2-buffered medium [11], whereas it is stimulated about four-fold by αKG preloading in HEPES-buffered medium [33]. Although metabolic production of αKG must also be important for PAH transport by snake renal tubules, the stimulation of such transport by preloading with αKG is not influenced by the buffer system used (W.H. Dantzler, unpublished observations). This effect has not been studied with renal tubules from any other species.

We have evaluated the relative roles of metabolism and uptake via the basolateral Na^+-dicarboxylate cotransporter

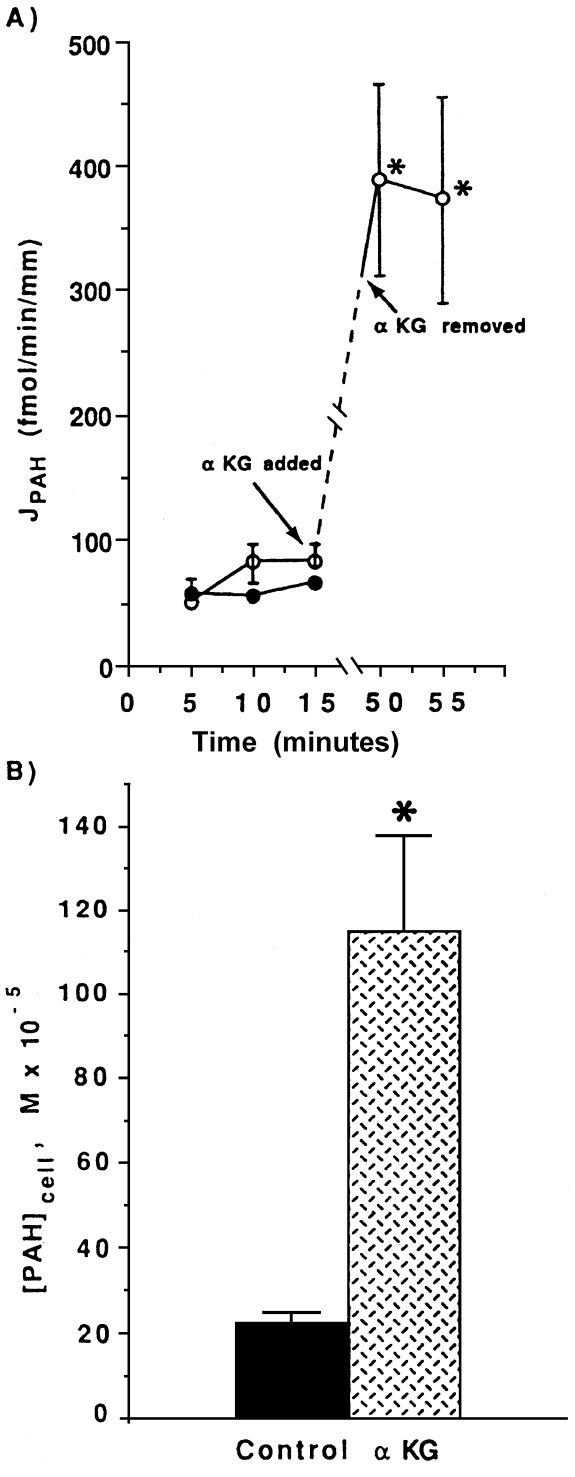

Fig. 2. Comparison of net transepithelial secretory transport of PAH (J_{PAH}) (A) and cell concentrations of PAH (B) between control and αKG-loaded snake proximal renal tubules from the same kidney studied in parallel. Results are means ± S.E. *Group means that are significantly ($P<0.05$) different from control values. From Ref. [32] with permission.

in establishing the outwardly directed gradient for αKG in nonperfused and perfused rabbit tubules under a close approximation to physiological conditions (bicarbonate/CO_2 buffer, the presence of other metabolic substrates, physiological concentration of αKG) [34,35]. In these studies, we examined transport in real time by using epifluorescence microscopy and observing the transport of FL, which is also transported by this general OA transport system, rather than PAH. Uptake of 1 μM FL by non-perfused rabbit proximal tubules is linear for the first 25 s and this initial rate of uptake is absolutely reproducible for at least 3 h (Fig. 3) [35]. When we examined the effects of preloading the tubules with αKG (*trans* configuration) on this initial rate of uptake, we found that a concentration of 10 μM in the bathing medium (approximately the normal plasma concentration) was as effective as 100 μM used

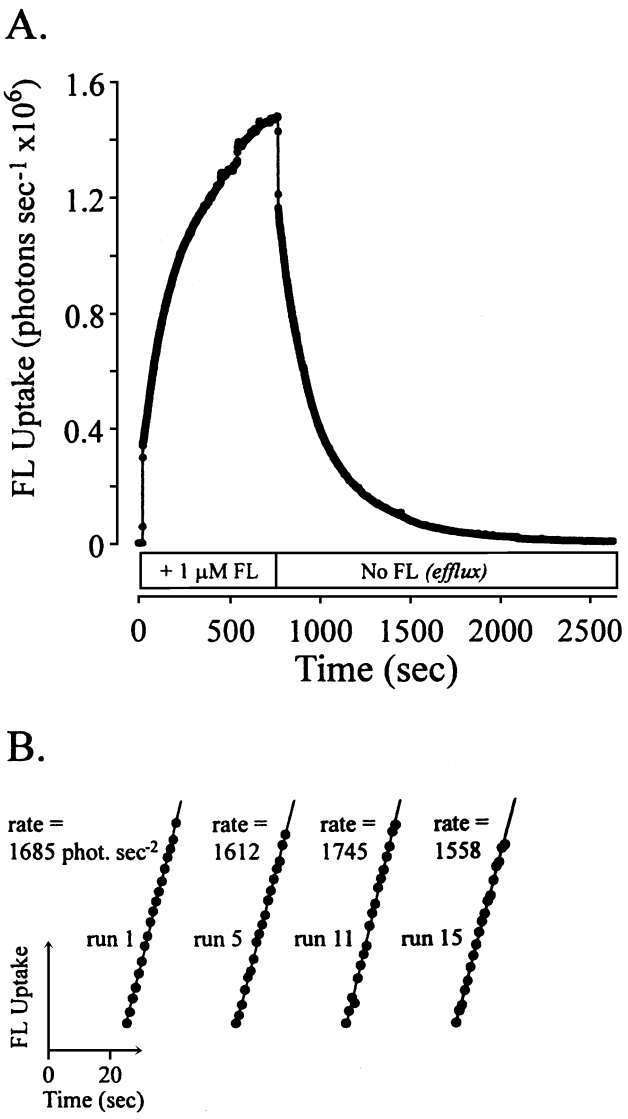

Fig. 3. (A) Fluorescence profile of an intact rabbit proximal S2 renal tubule exposed first to a superfusion buffer containing 1 μM FL (FL influx) and then to a buffer containing no FL (efflux). (B) Initial rates of FL uptake into an intact rabbit renal proximal S2 tubule measured using a brief-exposure protocol and a superfusion buffer containing 1 μM FL and 20 μM αKG in a *cis–trans* configuration. The 4 uptake rates shown are representative of 15 initial rates of FL uptake measured over a 3-h period. From Ref. [35] with permission.

previously in the studies noted above (Fig. 4) [35]. Since the *cis–trans* configuration for the presence of αKG (tubules preloaded in the presence of 10 μM αKG to establish the *trans* configuration and then maintained with 10 μM αKG

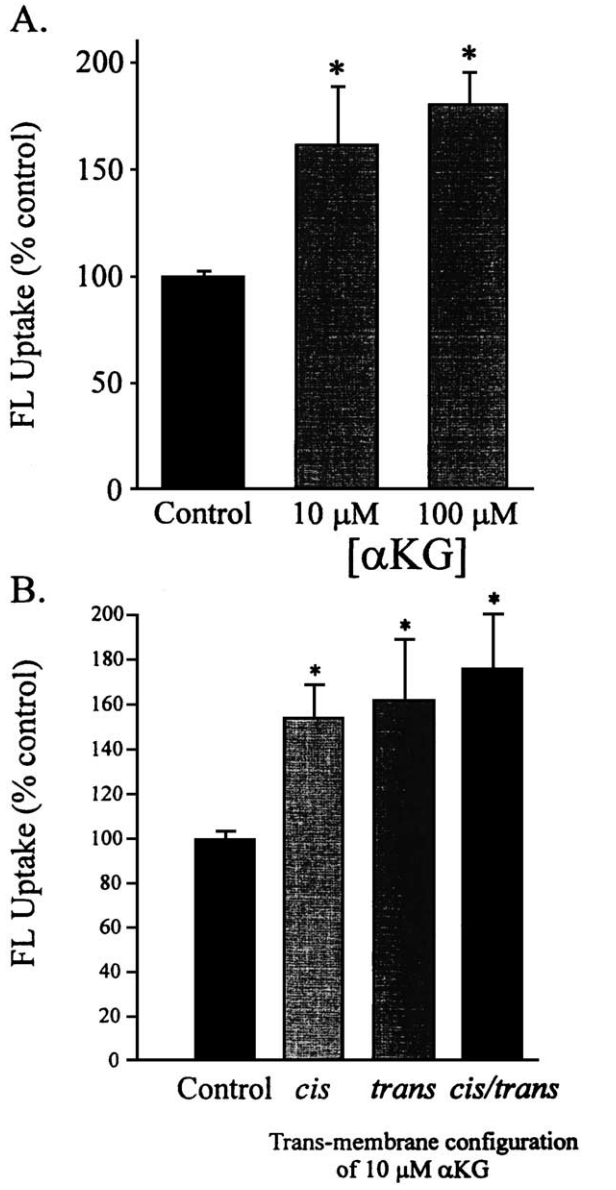

Fig. 4. (A) Stimulation of uptake of 1 μM FL into intact rabbit renal proximal S2 tubules resulting from exposure to 10 or 100 μM αKG in the *trans*-configuration. Each bar is mean ± S.E. ($n = 18$) of the initial rate of FL uptake compared with the average rate of uptake measured in αKG-free buffer (controls). *Mean initial rate of FL uptake that is significantly ($P = < 0.05$) different from paired controls. (B) Effect of *cis*, *trans*, and *cis–trans* configurations of 10 μM αKG on the initial rate of FL uptake into intact rabbit renal proximal S2 renal tubules. Tubules were exposed to each of the transmembrane configurations of 10 μM αKG prior to measurement of the initial rate of 1 μM FL uptake. Each bar is mean ± S.E. of initial rate of FL uptake in 8–11 tubules compared with the initial rate of uptake measured in absence of αKG. *Mean initial rate of FL uptake that is significantly ($P = < 0.05$) different from paired controls. From Ref. [35] with permission.

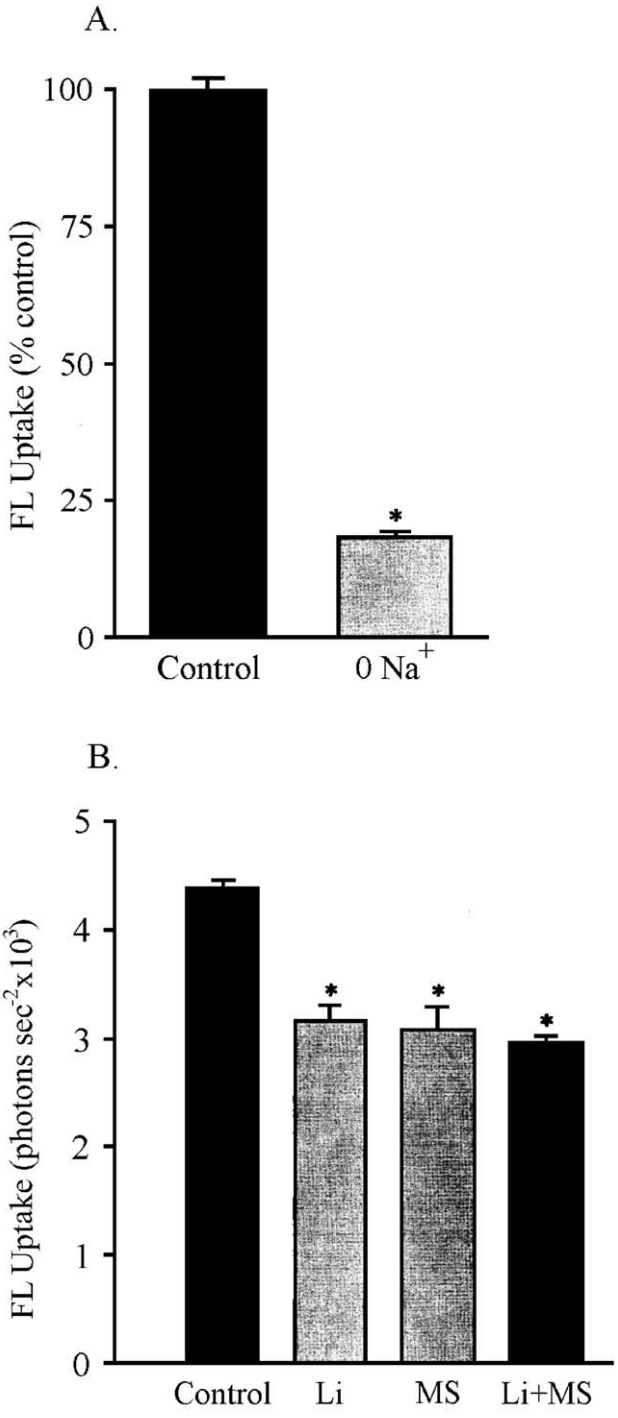

Fig. 5. (A) Effect of sodium removal on the initial rate of uptake of 1 μM FL into single rabbit renal proximal S2 tubules. Sodium in the experimental uptake medium was replaced with *N*-methyl-D-glucamine (NMDG). Buffers did not contain αKG. Each bar is mean ± S.E. of initial rate of FL uptake in five tubules. *Mean initial rate of FL that is significantly ($P < 0.05$) different from paired controls. (B) Effect of 2 mM LiCl and 1 mM methylsuccinate (MS) on the initial rate of uptake of 1 μM FL into single rabbit renal proximal S2 tubules. Buffers did not contain αKG. Each bar is mean ± S.E. of two to three sequential measurements of FL uptake in the presence of one or both of the test agents. Control bar is mean ± S.E. of nine measurements of initial rate of uptake measured before and after each of the test conditions. *Initial rate of FL uptake that is significantly ($P < 0.05$) different from controls. From Ref. [35] with permission.

on the *cis* or outside of the membrane to establish the *cis–trans* configuration) would represent the normal situation, we compared this configuration with the *cis* and *trans* configurations alone (Fig. 4) [35]. As shown in Fig. 4, there is no significant difference between these configurations, the average increase compared with control being ~75%. However, this lack of significant difference is partially due to the scatter in the experiments because all but one of the experiments in the *cis–trans* configuration showed more rapid uptake than experiments in the trans configuration alone [35]. We were also interested in the significance of recycling (by the Na^+-dicarboxylate cotransporter) of αKG that has moved out of the cells in exchange for an OA in maintaining an outwardly directed αKG gradient sufficient for continued OA uptake. To examine this question, we determined the effect of inhibiting the Na^+-dicarboxylate cotransporter (with no added αKG in the bathing medium) on the rate of FL uptake [35]. The results are shown in Fig. 5. Blocking the Na^+-dicarboxylate cotransporter by removing Na^+ from the bathing medium (replacement with *N*-methyl-D-glucamine) has a profound effect on FL uptake (Fig. 5). However, such a maneuver could have additional effects that could interfere with FL uptake. Therefore, we inhibited the Na^+-dicarboxylate transporter specifically exposing it to Li^+, by saturating it with a high concentration of methylsulfate (which is transported by this transporter but does not exchange for FL), or by a combination of the two. Both maneuvers have a significant inhibitory effect on FL uptake (but less than Na^+ replacement) and they are not additive (Fig. 5). The consistent observation that selective inhibition of the Na^+-dicarboxylate cotransporter in the absence of exogenous αKG reduces FL transport by ~25% suggests that ~25% of the peritubular OA/αKG exchange activity requires the parallel activity of the Na^+-dicarboxylate cotransporter.

Essentially the same response is observed if we measure not just the rate of basolateral uptake of FL but the transepithelial transport of FL in real time. We developed a method to make these measurements [34]. This involves epifluorescent microscopic measurement of the appearance of FL in the collected perfusate from isolated perfused tubules. Using this technique, we found that the addition of Li^+ to the bathing medium in the absence of exogenous αKG reduces net transepithelial transport of FL by ~25% (Fig. 6) [34]. This is the same degree of depression as seen with FL uptake at the basolateral membrane (see above). Thus, ~25% of basolateral OA/αKG exchange and the resulting transepithelial transport requires the parallel activity of the basolateral Na^+-dicarboxylate cotransporter.

From these studies, we have developed a quantitative estimate of the relative importance of metabolism and basolateral uptake via the Na^+-dicarboxylate cotransporter in establishing and maintaining the outwardly directed αKG gradient for basolateral OA/αKG exchange, at least for rabbit tubules (Fig. 1) [34,35]. Recycling of αKG across the peritubular membrane supports ~25% of the "basal" activity of the OA/αKG exchanger, i.e., the activity that is supported by cellular metabolism alone. However, the basolateral membrane at steady-state is also exposed to αKG in the *cis–trans* configuration. Under this configuration, αKG increases the rate of FL transport by ~75% over the basal rate, presumably by increasing the cytoplasmic concentration of αKG over that produced by metabolism alone. These two observations suggest that the basolateral Na^+-dicarbox-

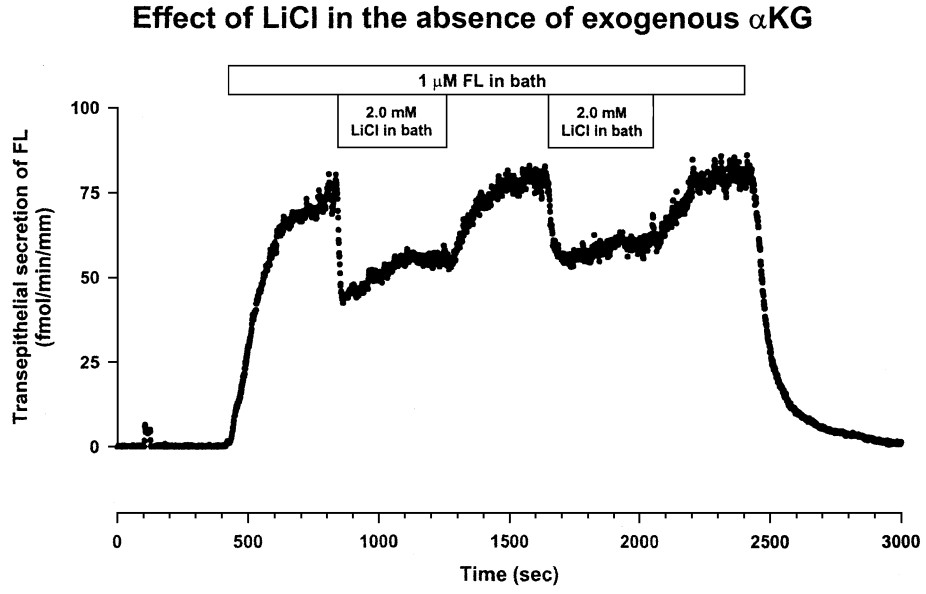

Fig. 6. Representative tracing of effect 2 mM LiCl in bathing medium on net transepithelial secretion of FL in fmol min^{-1} mm^{-1} by a single rabbit renal proximal S2 tubule in real time. FL concentration in bathing medium was 1 μM. Bars above tracing show periods when FL and LiCl were present in the bathing medium. Data are taken from the studies reported in Ref. [34].

ylate cotransporter is directly responsible for supporting ~ 57% [(75 + 25%)/175%] of the total basolateral transport of OA via the OA/αKG exchanger. This exchanger has been cloned by two groups essentially simultaneously from the rat kidney [36,37] and, slightly later, from flounder kidney [38] and mouse kidney [39]. Orthologs have also now been cloned from the human kidney [40,41] and, apparently, from the rabbit kidney (A. Bahn and G. Burckhardt, personal communication). It is generally referred to as OAT1 (for Organic Anion Transporter 1).

Of course, αKG, which is circulating in the blood, is filtered at the glomerulus and can be reabsorbed from the lumen via a Na^+-dicarboxylate cotransporter located on the luminal membrane (Fig. 1). In rabbit renal tubules, this luminal Na^+-dicarboxylate cotransporter, which has been cloned and sequenced [42], has a higher capacity than the basolateral Na^+-dicarboxylate cotransporter. We hypothesized that uptake of αKG via this luminal transporter might play a role in establishing the outwardly directed αKG gradient at the basolateral membrane and, therefore, in determining OA uptake across the basolateral membrane via OA/αKG exchange. To test this possibility, we initially examined the effect of adding αKG or glutarate (a non-metabolized dicarboxylate that is also countertransported for PAH at the basolateral membrane via OAT1) to the lumen of perfused rabbit and snake tubules on net PAH secretion ([43]; W.H. Dantzler and K.K. Evans, unpublished observations). The addition of 100 μM αKG or glutarate to the lumen (with none in the bathing medium) produces a doubling of net transepithelial PAH secretion (Fig. 7). Since this effect is the same with αKG and glutarate, it cannot be the effect of αKG metabolism and, thereby, increased energy production. When the luminal Na^+-dicarboxylate cotransporter is blocked by Li^+, this stimulation is completely eliminated, but it can still be elicited when Li^+ is removed from the lumen (Fig. 7). Unfortunately, the initial studies on rabbit tubules were performed under nonphysiological conditions (with HEPES-buffered media instead of bicarbonate/CO_2-buffered media and with high concentrations of αKG) [43]. At first, we could not repeat these under more physiological conditions, and we suggested that uptake of αKG from the lumen by the Na^+-dicarboxylate cotransporter only played a role in OA transport when metabolism was depressed [43]. However, when we reexamined this question under a close approximation of physiological conditions while measuring net transepithelial FL transport in real time, we found that luminal uptake of αKG is important [34]. The addition of ~ 20–50 μM αKG to the lumen alone (with none in the bathing medium) produces a significant increase in FL secretion of about 20% (Fig. 8) [34]. Of even greater importance, the presence of 50 μM αKG in the lumen in the presence of 10 μM αKG in the bath

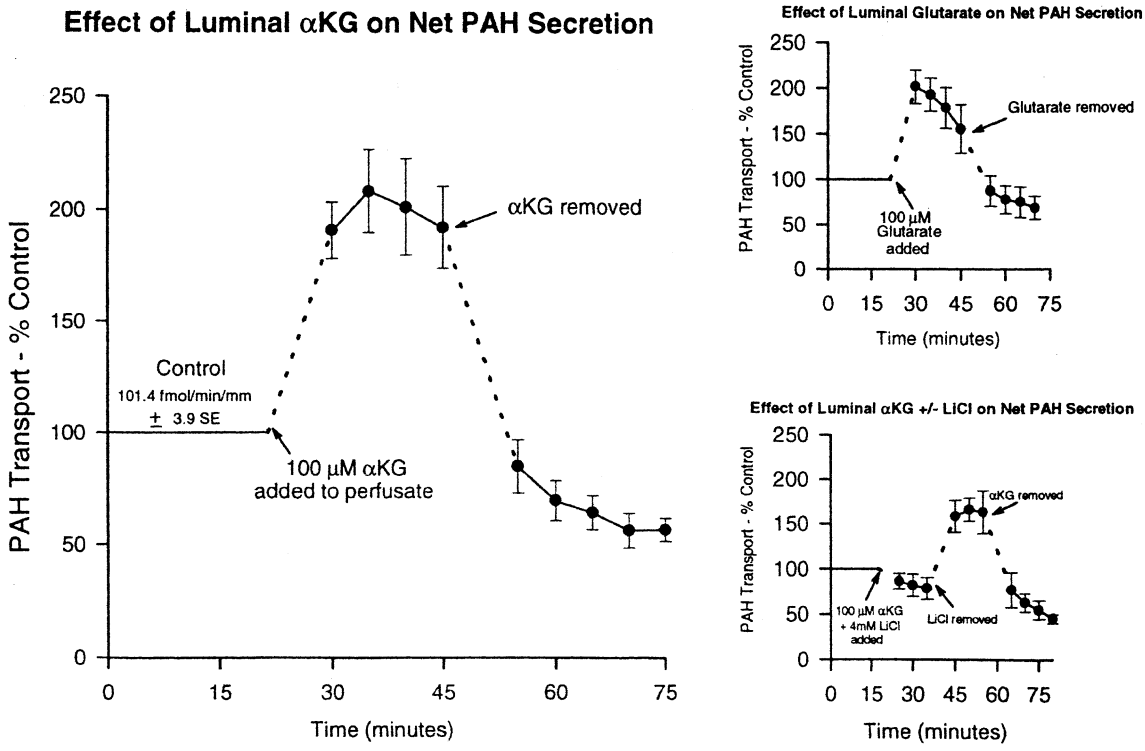

Fig. 7. Effects of 100 μM αKG or glutarate in perfusate on net transepithelial secretion (J_{PAH}) of PAH in rabbit renal proximal S2 tubules perfused and bathed in HEPES-buffered medium. Effect of 100 μM αKG in perfusate on J_{PAH} also shown in the presence and absence of 4 mM LiCl in perfusate. Values for J_{PAH} are shown as percent of the mean control value (control being 100%) for each tubule for the first 20 min. Absolute mean rate of J_{PAH} for all tubules for the first 20 min is given in fmol min^{-1} mm^{-1} (± S.E.). Solid circles are means of values for all tubules; vertical lines are ± S.E.; $n = 6$. Arrows indicate when substances were added to or removed from the perfusate. Data are taken from the studies reported in Ref. [43].

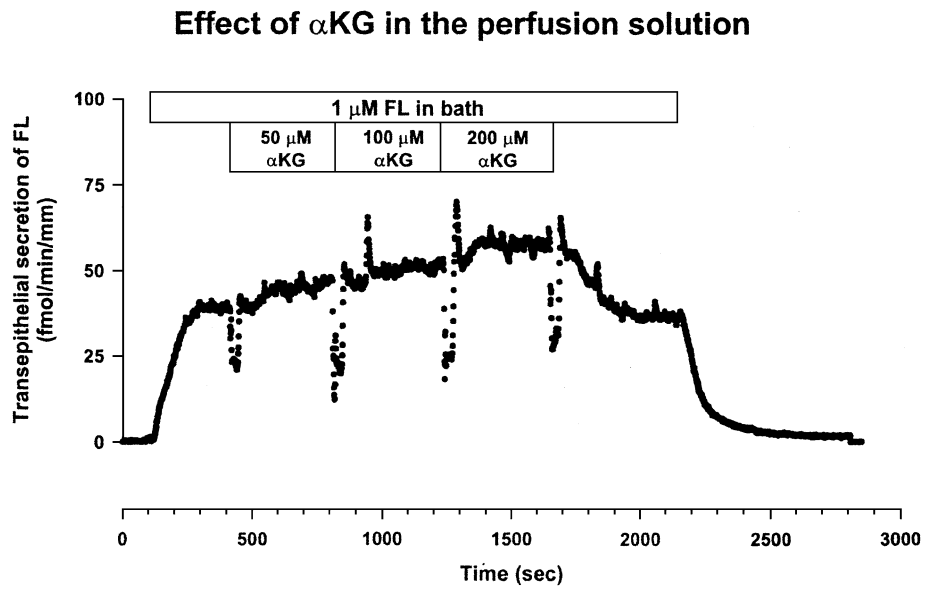

Fig. 8. Representative tracing of effect of addition of 50, 100, or 200 μM αKG to perfusate on net transepithelial secretion of FL in fmol min^{-1} mm^{-1} by a single rabbit renal proximal S2 tubule in real time. FL concentration in bathing medium was 1 μM. Bars above tracing show periods when FL and was present in bathing medium and αKG was present at various concentrations in luminal perfusate. Data are taken from studies reported in Ref. [34].

produces a significant increase of about 15% over that observed with αKG in the bathing medium alone (Fig. 9) [34]. Putting all these observations together, we suggest that, at least in rabbit tubules, the basolateral and luminal Na^+-dicarboxylate cotransporters together contribute about 60% to the αKG gradient and metabolism contributes about 40%. However, the luminal dicarboxylate transporter only contributes about 14% of the total contributed by the two dicarboxylate transporters (Fig. 1). Although the general model of basolateral transport shown in Fig. 1 appears to apply to fish, amphibian, reptilian, avian, and mammalian tubules, the data on the relative roles of metabolism and αKG transport in establishing the αKG gradient are only complete for mammalian tubules.

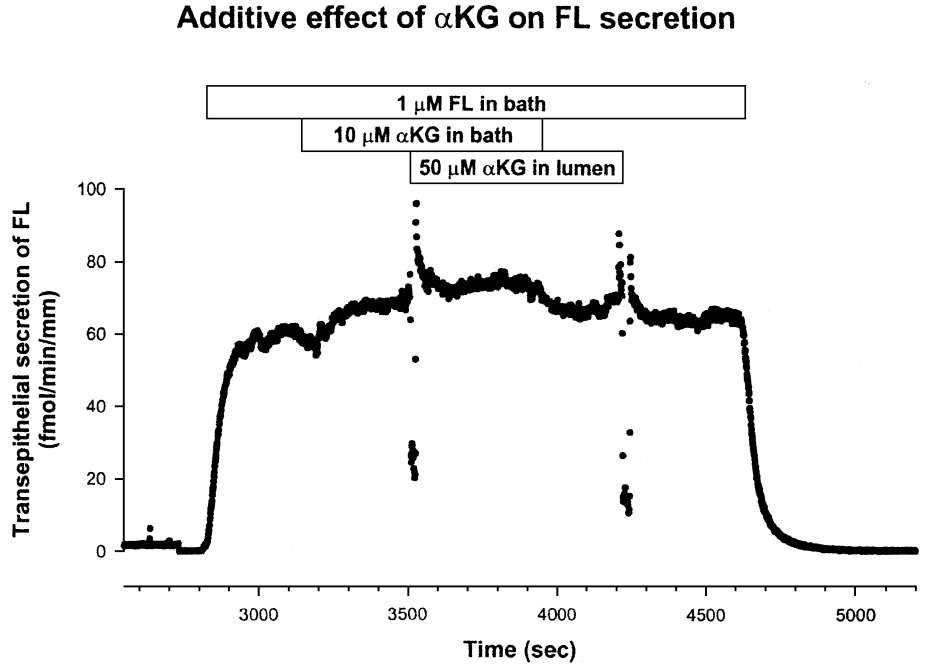

Fig. 9. Representative tracing of effect of addition of αKG to bathing medium, perfusate, or both on net transepithelial secretion of FL in fmol min^{-1} mm^{-1} by a single rabbit renal proximal S2 tubule in real time. FL concentration in bathing medium was 1 μM. Concentration of αKG was 10 μM in bathing medium and 50 μM in perfusate. Bars above tracing show when FL and αKG were present in bathing medium and when αKG was present in luminal perfusate. Data are taken from studies reported in Ref. [34].

4. Regulation of basolateral transport

Is this classical transport system for OAs at the basolateral membrane regulated physiologically? Until quite recently, this question had never been considered. However, given the importance of this system in regulating the concentrations of numerous endogenous organic compounds and in eliminating toxic or potentially toxic xenobiotics, some form of regulation appears reasonable. Sequence data on the cloned OAT1 reveal a number consensus sites for phosphorylation of the transporter protein by protein kinase C (PKC). This suggested that regulation could involve activation of PKC. Indeed, before the transporter was cloned, Hohage et al. [44] reported that PAH uptake by isolated rabbit tubules was stimulated by activation of PKC with phorbol esters. In contrast, however, Miller [45] and Takano et al. [46] reported inhibition of OA uptake by PKC activation in killifish proximal tubules and opossum kidney (OK) cells, respectively. We reexamined the question by evaluating the effects of PKC activation on the initial rate of FL uptake in isolated nonperfused rabbit tubules [47] and the transepithelial secretion of FL in isolated perfused rabbit tubules [48]. We used four different maneuvers, all known to lead to an activation of PKC in the proximal tubule: addition to the bathing medium of (1) 4-β-phorbol 12-myristate 13-acetate (PMA); (2) 1,2-*sn*-dioctanylglycerol (DOG); (3) the α_1-receptor agonist phenylephrine (PE); or the peptide hormone bradykinin (BK). Both PE and BK stimulate PKC via a ligand–receptor–PKC coupling reaction. In the nonperfused tubules, the addition of PMA or DOG to the bathing medium induces a rapid and dramatic decrease in basolateral FL uptake which is not reversible (Fig. 10) [47]. We obtained a similar result on transepithelial secretion of FL with perfused tubules [48]. Both BK and PE also significantly decrease FL uptake in nonperfused tubules (Figs. 11 and 12) [47] and transepithelial FL secretion in perfused tubules [48], but these effects are reversible when these agents are removed from the bathing medium. To be certain that the observed effects were indeed predominantly due to PKC activation, we employed the PKC inhibitor bisindolylmaleimide I (BIM) in a concentration at which no other effects have been described. BIM reduces the inhibitory effects of PMA, BK, and PE in nonperfused (Fig. 13) [47]

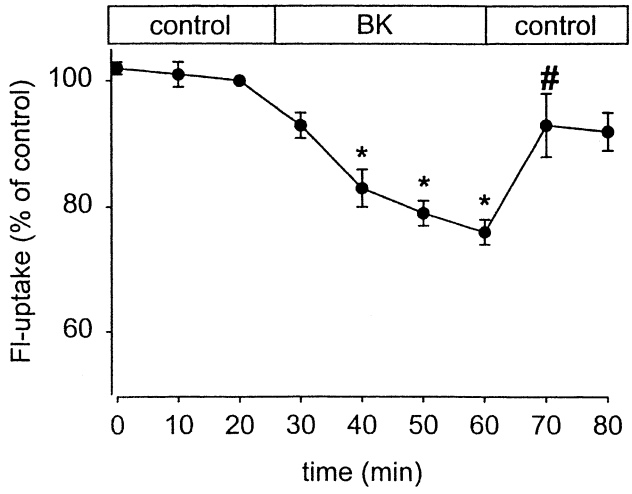

Fig. 11. Effect of BK (10^{-6} M) on initial rate of FL (1 μM) uptake in intact rabbit renal proximal S2 tubules. All values are normalized to the last control measurement. Symbols are means ± S.E. for each value plotted ($n=6$). *$P<0.05$ vs. last control value; #$P<0.05$ vs. last value in presence of BK (from Ref. [47] with permission).

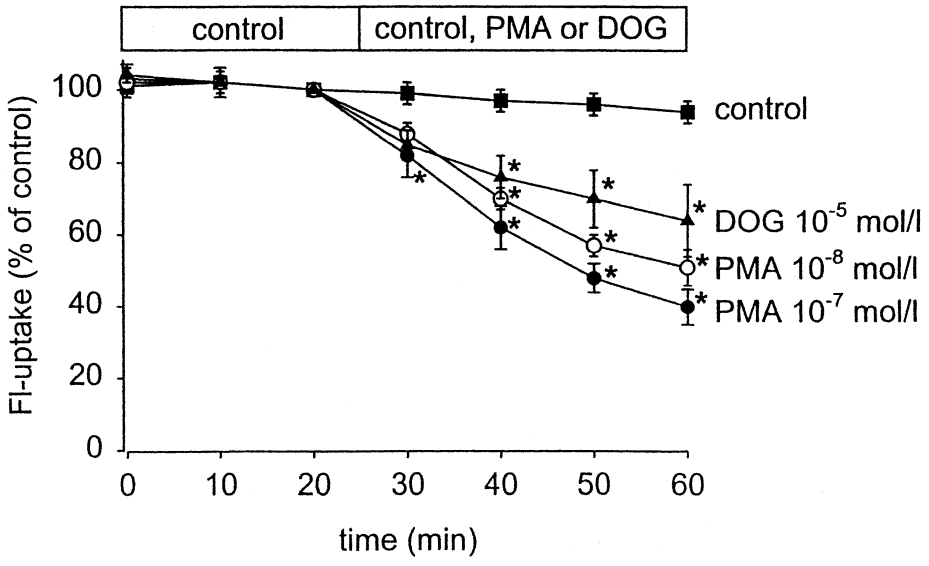

Fig. 10. Effect of PKC stimulation with PMA or DOG on initial rate of FL uptake in intact rabbit renal proximal S2 tubules. All values are normalized to the last control measurement. Control contains only the vehicle (dimethylsulfoxide; final concentration: ≤ 1:10,000) in which PMA or DOG was dissolved. Symbols are means ± S.E. for each value plotted ($n=6$). *$P<0.05$ vs. last control measurement. From Ref. [47] with permission.

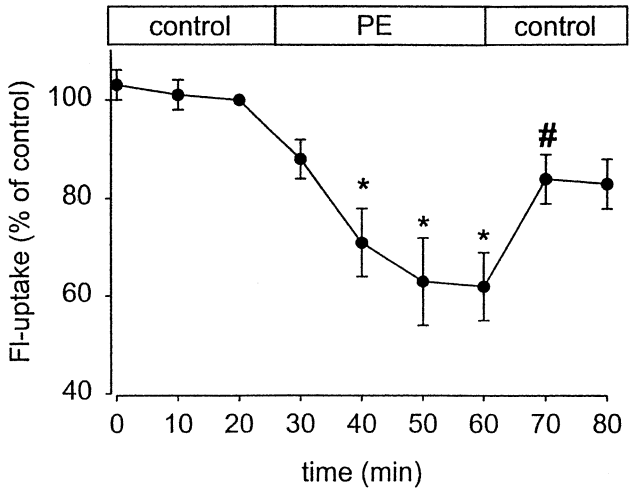

Fig. 12. Effect of PE (10^{-6} M) on initial rate of FL (1 μM) uptake in intact rabbit renal proximal S2 tubules. All values are normalized to last control measurement. Symbols are means ± S.E. for each value plotted ($n=6$). *$P<0.05$ vs. last control value; #$P<0.05$ vs. last value in presence of BK. From Ref. [47] with permission.

and perfused tubules [48]. In addition, we showed that PMA does not work by inhibiting αKG production in the cells because it is even more effective in reducing FL uptake by nonperfused tubules when the tubules are supplied with sufficient exogenous αKG to keep the OA/αKG exchanger not only minimally supplied but stimulated [47]. Thus, there is now evidence that this transporter is regulated by physiological stimuli (e.g., hormones or the autonomic nervous system) in intact tubules via activation of PKC. However, as suggested by recent work on the cloned mouse OAT1 expressed in LLC-PK_1 cells, this may not involve direct phosphorylation of mOAT by PKC [49].

Other recent data indicate that epidermal growth factor (EGF), which appears to be important in normal tubulogenesis or tubular regeneration after injury, can enhance basolateral uptake of OA in OK cells, which normally contain the OA transport system [50]. This effect, in the OK cell line, appears to occur via the mitogen-activated protein kinase (MAPK) pathway because it is abolished by inhibition of mitogen-activated/extracellular signal-regulated kinase kinase (MEK) and because administration of EGF leads to phosphorylation of extracellular signal-regulated kinase 1 and 2 (ERK 1/2) [50]. Our preliminary data also indicate that EGF stimulates PAH uptake by intact rabbit tubules and that this stimulation is eliminated by inhibition of MEK [51]. Therefore, at least a number of physiological factors appear to regulate the basolateral OA transporter by a number of phosphorylation pathways.

5. Luminal transport

During the process of net transepithelial secretion of OAs, they move from the cells to the tubule lumen down their electrochemical gradient (Fig. 1). This process must be mediated in some fashion to account for the relatively high apparent permeability of the membrane to substances with relatively poor lipid solubilities (Table 2). However, this transport process is much less well understood than that at the basolateral membrane. Indeed, the basic process may differ between vertebrate classes and between species within a given vertebrate class.

A number of studies of this process have been made in fishes, reptiles, and mammals. Studies with flounder brush border membrane vesicles (BBMV) suggest that this process involves carrier-mediated diffusion driven by the electrical gradient [20], but other work with intact flounder tubules does not support this observation (D.S. Miller, personal communication). We have studied the process with isolated, perfused snake renal tubules. In these tubules, movement of PAH from the cells to the lumen is inhibited by SITS [24], suggesting that it may involve anion exchange. However, in contrast to what would be expected in this case, the movement of radiolabeled PAH into the lumen is inhibited by unlabeled PAH or phenol red in the lumen rather than stimulated [52]. Of course, anion exchange could still be occurring but the loaded carrier be translocating more

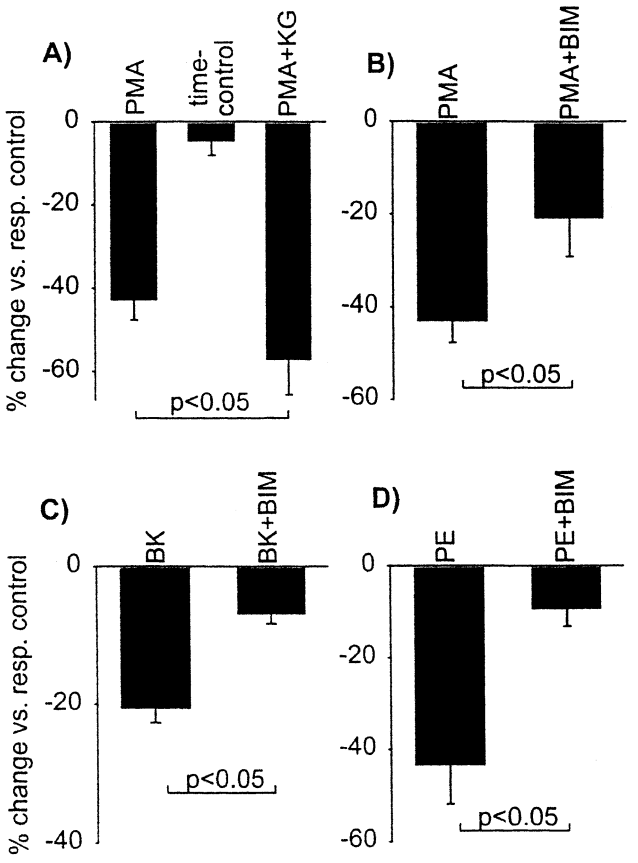

Fig. 13. Effect of PKC inhibitor BIM (10^{-7} M) on inhibition of initial rate of FL (1 mM) uptake produced by PMA, BK, or PE. Columns show change in FL uptake after 25 min exposure vs. last control value under different conditions. Values are means ± S.E. ($n=6$). resp., respective. Modified from figures in Ref. [47] with permission.

slowly than the unloaded carrier. The movement of PAH into the lumen is not dependent on the presence of chloride in the lumen, but substitutions for chloride suggest that it may be dependent on the presence of an anion in the lumen to which the membrane is highly permeable [53]. This could be further evidence for anion exchange or it could result from an effect on membrane potential. Of particular interest in snake tubules is the observation that anything that inhibits PAH movement from the cells to the lumen secondarily results in a reduction in PAH uptake across the basolateral membrane, suggesting some type of feedback system between these two membrane transporters (Fig. 1) [24,52,53].

In mammals, the situation is even more confusing because there appear to be many variations among species. For example, studies with BBMV from species whose proximal tubules reabsorb urate (e.g., dogs and rats) give evidence for an anion exchanger that can transport PAH (and numerous other anions), but that appears to be poised in the direction of reabsorbing urate [54–59]. Moreover, this exchanger appears to be absent in BBMV from rabbits and pigs, species whose kidneys secrete urate [54,59,60,61]. Indeed, there is some evidence from rabbit and pig BBMV for carrier-mediated diffusion driven by the membrane potential [60,61,62]. In addition, studies with bovine and human BBMV even provide some evidence for a PAH/αKG exchanger in this membrane [63,64], although how two exchangers of the same type in both membranes might be poised to produce net transport of OAs from peritubular side to luminal side of the tubule is not clear. Finally, there is some evidence that the rabbit luminal Type I Na^+-phosphate cotransporter (NaPi-1) (a weak phosphate co-transporter) may function as an electrogenic carrier or channel for OAs when expressed in *Xenopus* oocytes [65].

It is obvious that very little is understood about this luminal transport step. We are approaching this problem in two ways. First, we have designed a system that makes it possible for us to examine transport of FL from the cells to the lumen across this membrane in real time [66]. The method involves efflux of FL across the luminal membrane of a perfused tubule in real time. Initial data with this system are shown for a rabbit tubule in Fig. 14. These data show clearly that the transport step saturates. The kinetic analysis of these data [66] when compared with the analysis for net transepithelial transport [34] support the concept that the transport step at the basolateral membrane is rate-limiting for net secretion.

Second, we are approaching this problem from a molecular direction. One of the OAT family of OA transporters isolated from the rat and human kidneys, OAT4, appears to be located at the luminal membrane of the proximal tubule, at least in humans [67]. Moreover, although this isolated protein, when expressed in *Xenopus* oocytes, supports PAH transport, it apparently does not support PAH/αKG exchange or PAH transport against an electrochemical gradient [67]. Therefore, it seems to be a potential candidate for a luminal transporter. We are working to express both OAT4 and OAT1 in the same cell system to determine if, together, they can support transepithelial transport.

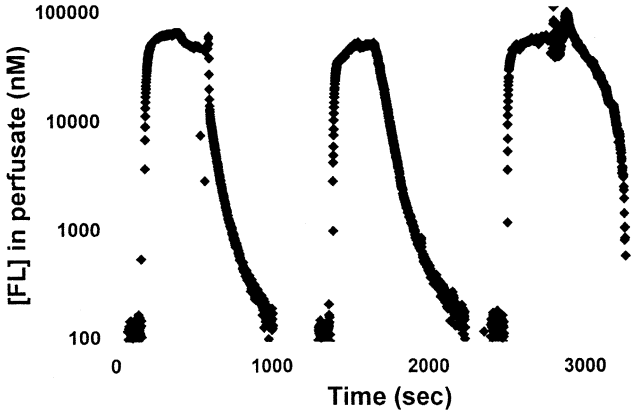

Fig. 14. Sample tracing showing consecutive washout profiles of FL obtained from a single rabbit renal proximal S2 tubule in real time. The concentration of FL in the collected perfusate is indicated on a log scale on the ordinate. Time is indicated on a linear scale on the abscissa. The tubule was exposed to 50 mM FL until a steady-state was obtained. FL was then removed from the bathing medium and the disappearance of FL from the collected perfusate (reflecting efflux) was observed. In the first two profiles, the bathing medium remained standard bicarbonate-buffered medium when FL was removed from it. Under these circumstances, efflux of FL from the tubule occurs across both the luminal and basolateral membranes. In the third profile, the bathing medium was simply replaced by mineral oil. With the oil-covered tubule, efflux occurs only across the luminal membrane. In this case, the luminal efflux is clearly shown to saturate and kinetic constants can be determined from the saturation curves. Modified from Ref. [66] with permission.

6. Intracellular transport

Most people have assumed that OAs, such as FL or PAH, transported into the renal tubule cells at the basolateral membrane diffuse through the cells to the luminal membrane during the process of net transepithelial transport. In recent years, however, some questions have been raised about this assumption. Indeed, early studies had suggested that intracellular binding of other transported substrates, such as probenecid and phenol red, might occur [68,69], but this was not supported by further studies [70]. More recently, Miller and Pritchard and their co-workers [71,72] using epifluorescence video-imaging and confocal microscopy techniques to study transepithelial transport of FL in flounder renal tubules, crab urinary bladder, and OK cells have obtained evidence for accumulation of this OA in punctate compartments (possibly vesicles). We have performed some similar preliminary studies with isolated renal tubules from rabbit, snake, and chicken kidneys ([73]; S. Shpun and W.H. Dantzler, unpublished observations). Our initial studies performed with a Tris-buffered medium identical to that used by Miller et al. [71] indicated that FL does accumulate in punctate compartments in the cells of isolated

rabbit proximal S2 tubules (and possibly in the cells of snake and chicken proximal tubules). However, with the rabbit tubules in this bicarbonate-free buffer, as might be expected from our observations about buffers noted above, very little FL appears in the tubule lumens. When the medium bathing the tubules is replaced with a bicarbonate/CO_2-buffered one (with or without Tris), FL appears in the lumen and no longer appears in punctate compartments in the cytoplasm. We have not yet examined the effect of different buffers in tubules from nonmammalian vertebrates, but judging by the lack of effect of different buffers on OA transport in snake tubules (see above), it may not make a difference in these species. In any case, it appears that in mammals, cellular metabolism is important in determining the degree of accumulation in intracellular compartments and that such accumulation may not be a factor in transport under physiological conditions.

7. Conclusions

In all those vertebrates studied, the general system for the transepithelial renal secretion of a wide range of hydrophobic OAs involves transport into the cells of the proximal tubules against an electrochemical gradient at the basolateral membrane by countertransport for αKG moving down its electrochemical gradient (Fig. 1). This is the dominant, rate-limiting step in the transepithelial secretory process. The outwardly directed gradient for αKG at the basolateral membrane is maintained by metabolism and by uptake across both the basolateral and luminal membranes by Na^+-dicarboxylate cotransporters (Fig. 1). In mammals, but not in nonmammalian species, we now have information on the relative importance of these processes in maintaining the gradient. We also now know that this basolateral transport step is regulated under physiological conditions and we are beginning to obtain information about several apparent regulatory systems in mammals. These include peptide hormones (e.g., BK) and the autonomic nervous system working via PKC pathways and EGF working via the MAPK pathway.

Within the cells, movement of these OAs to the luminal side may involve diffusion, especially in mammalian tubules under physiological conditions. However, under some circumstances in mammalian tubules (e.g., when the metabolic state is compromised) and possibly more generally in nonmammalian tubules, it may involve accumulation in some form of vesicles, which then move across the cells (Fig. 1).

Movement of OAs from the cells to the lumen across the luminal membrane is clearly mediated in some fashion (Fig. 1), but the nature of such mediated transport is not adequately understood for any species. Indeed, this transport step may vary among vertebrate classes and among species within any one vertebrate class. We now have preliminary direct evidence on the saturation kinetics of this transporter in rabbit proximal tubules and are working on at least one possible molecular mechanism.

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