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Review

The molecular and cellular physiology of basolateral organic anion transport in mammalian renal tubules

William H. Dantzler*, Stephen H. Wright

Department of Physiology, Health Science Center, College of Medicine, University of Arizona, Tucson, AZ 85724-5051, USA

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Abstract

Basolateral transport of organic anions (OAs) into mammalian renal proximal tubule cells is a tertiary active transport process. The final step in this process involves movement of OA into the cells against its electrochemical gradient in exchange for α-ketoglutarate (αKG) moving down its electrochemical gradient. Two homologous transport proteins (OAT1 and OAT3) that function as basolateral OA/αKG exchangers have been cloned and sequenced. We are in the process of determining the functional distribution and regulation of OAT1 and OAT3 in renal tubules. We are using rabbit OAT1 (rbOAT1) and OAT3 (rbOAT3) expressed in heterologous cell systems to determine substrate specificity and putative regulatory steps and isolated rabbit proximal renal tubule segments to determine functional distribution and physiological regulation of these transporters within their native epithelium. Rabbit OAT1 and OAT3 differ distinctly in substrate specificity. For example, rbOAT1 has a high affinity for the classical renal OA transport substrate, *p*-aminohippurate (PAH), whereas rbOAT3 has no affinity for PAH. In contrast, rbOAT3 has a high affinity for estrone sulfate (ES), whereas rbOAT1 has only a very slight affinity for ES. Both rbOAT1 and rbOAT3 appear to have about the same affinity for fluorescein (FL). These differences and similarities in substrate affinities make it possible to functionally map transporters along the renal tubules. Initial data indicate that OAT1 predominates in S2 segments of the rabbit proximal tubules, but studies of other segments are just beginning. Transport of a given substrate in any tubule segment depends on both the affinity of each transporter which can accept that substrate as well as the level of expression of each of those processes in that particular tubule segment. Basolateral PAH transport (presumably OAT1 activity) appears to be down-regulated by activation of protein kinase C (PKC) and up-regulated via mitogen-activated protein kinase (MAPK) through phospholipase A_2 (PLA_2), prostaglandin E_2 (PGE_2), cyclic AMP, and protein kinase A (PKA) activation.

Keywords: Renal tubule; Organic anion; Basolateral transport; OAT1; OAT3

1. Introduction

A large and diverse number of organic anions, or weak organic acids that exist as anions at physiological pH (collectively OAs), are secreted by mammalian renal tubules [1,2]. Substrates for the renal OA transport system include weak acids that have a net negative charge on carboxyl or sulfonyl residues at physiological pH. Although a number of endogenous OAs have been shown to be actively secreted by the proximal tubule (e.g., 5-hydroxyindoleacetic acid, riboflavin), it is generally accepted that the principal function of this process is clearing the body of xenobiotic agents [1,2], including many of the products of Phase I and Phase II hepatic biotransformation, as well as anionic drugs of therapeutic or recreational use [3–5].

Classical studies with isolated rabbit renal proximal tubules that examined the axial tubular distribution of secretion of the prototypical OA, *p*-aminohippurate (PAH), indicated that the S2 segment of the proximal tubule is the primary tubule site of OA excretion, although net PAH secretion certainly occurs in both the S1 and S3 segments [6–8]. Most kinetic studies with single tubules suggest that the difference in transport between these segments reflects a difference in the number of transporters per unit length rather than a difference in specific transporters [8,9]. However, some studies with transport inhibitors suggest that there might yet be some difference in the characteristics of the transporters between the S2 and S3 segments [8]. Different transporters with different substrate specificities

* Corresponding author. Tel.: +1-520-626-7646; fax: +1-520-626-2382.
E-mail address: dantzler@u.arizona.edu (W.H. Dantzler).

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in the different tubule segments could be extremely important in the kidney's ability to excrete specific xenobiotic agents under pathological conditions. They could also play a role in determining which segments are affected most by a given nephrotoxic xenobiotic agent. We now know that this is a much more likely possibility than previously thought (see below).

The transepithelial secretory process involves OA transport into the cells against an electrochemical gradient across the basolateral membrane and transport from the cells into the tubule lumen down an electrochemical gradient across the luminal membrane. Until recently, models of renal OA secretion typically depicted a single basolateral entry step and a single luminal exit step, a simple view that effectively explained existing physiological data. That view is now known to be an oversimplification of the suite of cellular events that underlies renal OA transport. At least two broad general secretory pathways can be recognized based on selectivities for two general substrate classes defined on the basis of molecular weight, net charge, and hydrophobicity. Adapting the classification scheme used to functionally categorize organic cation transporters [10], we will refer to the first group as the one handling 'Type I' OAs, which includes comparatively small (generally <400 MW) monovalent compounds, including PAH, probenecid, and fluorescein (FL). These are transported by what has been referred to as the "classical" OA secretory pathway. The second group of compounds ('Type II' OAs) is generally bulkier (typically >500 MW) and frequently polyvalent. They include, for example, leukotriene C_4 and estradiol-17β-D-glucoronide (E17β-D). We are concerned in this paper only with the "classical" pathway and the transport of "Type I" OAs. However, this pathway alone involves multiple transporters that can be functionally distinguished, at least in part, on the basis of the substrates with which they most efficiently interact (see below). The tubule location and regulation of these different transporters is likely to play a critical role in the excretion of specific xenobiotics under various physiological and pathological conditions.

The basolateral entry step for Type I OAs via the "classical" pathway is against an electrochemical gradient and, thus, involves an active, energy-requiring process. Initial studies with rat basolateral membrane vesicles [11–13] followed by studies with intact rabbit proximal tubules [14] convincingly demonstrated that the basolateral step in the "classical" pathway involves a tertiary active transport process (three basolateral transport processes arranged in parallel) (Fig. 1). The final step in the overall process is the countertransport of the OA (e.g., PAH or FL) into the cell against its electrochemical gradient in exchange for an intracellular dicarboxylate (physiologically, α-ketoglutarate (αKG)) moving down its electrochemical gradient (Fig. 1). The outwardly directed gradient for αKG is maintained in turn by metabolism (~ 40%) and by transport into the cells across both the basolateral and luminal membranes by the activity of Na^+-dicarboxylate

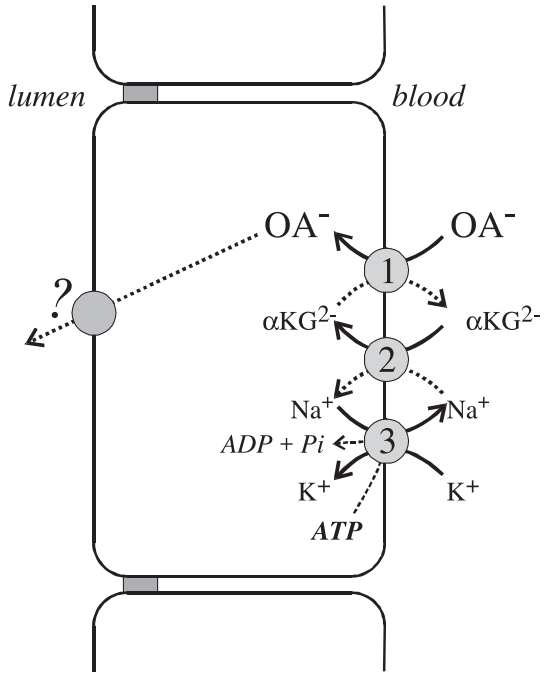

Fig. 1. Simplified descriptive model of transepithelial transport of OAs in proximal renal tubules. Transport of OA into cells at basolateral membrane involves a tertiary active transport process (three transport steps functioning in parallel). Solid arrows indicate transport against an electrochemical gradient. Broken arrows indicate transport down an electrochemical gradient. Question mark indicates uncertainty about nature of transporter. OA^- stands for any Type I organic anion transported by the classical pathway. αKG^{2-} stands for α-ketoglutarate. (1) $OA^-/\alpha KG^{2-}$ countertransport step, the final step in the tertiary active transport process that moves OA^- into cells. Molecular candidates for this step are OAT1 and OAT3. (2) Na^+-DC cotransport step that moves αKG^{2-} into cells. The primary molecular candidate for this step is Na-DC3. (3) Na^+-K^+-ATPase, the primary energy-requiring transport step that establishes and maintains the Na^+-gradient that drives step 2.

(Na-DC) cotransporters (~ 60%) [15,16] (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 (Fig. 1). Molecular candidates for each of these processes have been cloned. However, in this paper, we are concerned only with the basolateral OA/αKG exchange step, the critical final step in getting OAs into the cells across the basolateral membrane. We are primarily interested in determining the tubular distribution, relative functional significance, and regulation of the molecular candidates for this transport step. It is imperative that the functional distribution and regulation of these molecular transporters be determined within a native intact renal tubule epithelium. Among mammalian proximal tubules, only those from rabbit kidneys can be readily teased out without the aid of enzymatic agents for these determinations. Therefore, we have also cloned and begun characterizing the molecular candidates for this transport step from rabbit renal tissue (see below). We review our progress to date in these studies.

2. Molecular candidates for OA/αKG countertransport

The basolateral OA/αKG transporter appears to involve (at least) two distinct processes: OAT1 and OAT3. Two homologous transporters have been identified and also localized to the kidney (OAT2 and OAT4), but their roles in renal OA transport remain speculative. OAT1 was first cloned from rat kidney and functionally identified as an organic anion transporter in 1997 by two independent groups (OAT1 [17]; ROAT1 [18]). It proved to be the rat ortholog of a protein first cloned from mouse kidney, originally named NKT, for which no function was identified [19]. In addition to rat and mouse OAT1, orthologs have been cloned from human [20–23], flounder [24], and *C. elegans* [25], and recently from rabbit by us [26]. The mammalian orthologs display approximately 80% sequence identity with one another. The cDNAs for the four cloned mammalian OAT1s have open reading frames that code for proteins that range from 545 to 551 amino acids in length. Strong sequence homology with a group of previously cloned organic cation transporters led to the combined classification of the entire group of 'OCTs' and 'OATs' into a common family of transport proteins collectively referred to as the Organic Cation Family of transporters (alternatively referred to as the Amphiphilic Solute Facilitator Family [27]) that are, in turn, classified within the larger Major Facilitator Superfamily of transporters [28]. Conserved motifs within all OAT1 orthologs include three protein kinase C (PKC) consensus sites; and as many as five consensus sites for protein kinase A (PKA). Because we are working with isolated rabbit proximal tubules (as the standard model for studies of integrated function in isolated intact tubules because human tubules are not routinely available), we are concerned primarily with the rabbit OAT1 (rbOAT1).

OAT3 was also initially cloned from rat kidney, and its cDNA was found to encode for a protein 536 amino acids long [29]. Two OAT3 orthologs have been cloned from human kidney, only one of which supports OA transport [22,30]. This ortholog, hOAT3, codes for a 543 amino acid protein [30]. We have now cloned the rabbit ortholog (rbOAT3), the cDNA for which encodes for a 542 amino acid protein that shows 85% and 82% identity to the human and rat orthologs, respectively.

The general secondary structure of the OCT family of transporters, including OAT1 and OAT3, shows 12 transmembrane domains (TMD), intracellular N- and C-termini, a very long extracellular loop between TMD1 and TMD2 (which typically includes three to four N-linked glycosylation sites), and a long intracellular loop between TMD6 and TMD7. As noted above, all members of the family also have various numbers of PKC and PKA consensus sites.

The tubular distribution of OAT1 and OAT3 is far from clear. Immunocytochemical studies in rats suggest that OAT1 is located primarily in the basolateral membrane of cells in the S2 segment, although it is also found in the S1 and S3 segments [31,32]. This distribution is consistent with the previous physiological data on rabbit tubules showing the greatest PAH secretion in the S2 segment [6–8], but no direct quantitative information on functional distribution of OAT1 is available for these or any other renal tubules. Immunocytochemical data on human and rat kidneys suggest that OAT3 is expressed at the basolateral membrane of S1, S2, and S3 segments of the proximal tubule [31–33]. In the human kidney, expression levels in the proximal tubule appear to be in the order S1>S2 = S3 [31]. Again, however, no quantitative functional data on the distribution of this transporter are available. As indicated above, we are now in the process of determining the functional distribution and regulation of OAT1 and OAT3, the two known molecular candidates for the OA/αKG exchanger, in rabbit proximal renal tubules.

3. Substrate-specificity of OAT1 and OAT3

We have been comparing the substrate-specificity and transport characteristics of rbOAT1 and rbOAT3 with the two transporters expressed both artificially in heterologous expression systems and naturally in intact, isolated S2 segments of rabbit proximal renal tubules. For these studies, we transiently expressed rbOAT1 in COS7 cells and stably expressed rbOAT3 in CHO-K1 cells.

3.1. OAT1

We determined the kinetics of PAH transport by rbOAT1 by measuring radiolabeled PAH uptake into COS7 cells expressing rbOAT1 at increasing concentrations of unlabeled PAH [26]. PAH uptake was nearly linear for 2 min and that incubation time was used to provide an estimate of the initial rate of transport. As shown in Fig. 2, PAH transport saturated with a K_t of ~ 16 μM. To compare PAH transport by isolated rbOAT1 with transport by this process in its native intact epithelium, we made similar kinetic measurements of radiolabeled PAH uptake in isolated S2 segments of rabbit proximal tubules. The kinetics in the isolated tubules were similar to those seen in the COS7 cells expressing the transporter but the K_t was about four times higher (~ 70 μM) (Fig. 2). This difference in the K_ts between the two systems could reflect functional differences in the 'transport environment' of the two cell types in question (e.g., different regulatory profiles or the influence of unstirred layers on substrate availability in the intact tubule); or the potential influence of PAH interaction with additional transporters, including OAT3 transporter (but see below).

To be certain that the cloned transporter, rbOAT1, when expressed in COS7 cells actually functioned as an OA/αKG exchanger, we preloaded the transfected COS7 cells with glutarate (which can substitute for αKG in the exchange process) and measured the uptake of FL in real time. As shown in Fig. 3, preloading the cells with glutarate more than doubled the initial rate of FL uptake. Essentially, this is

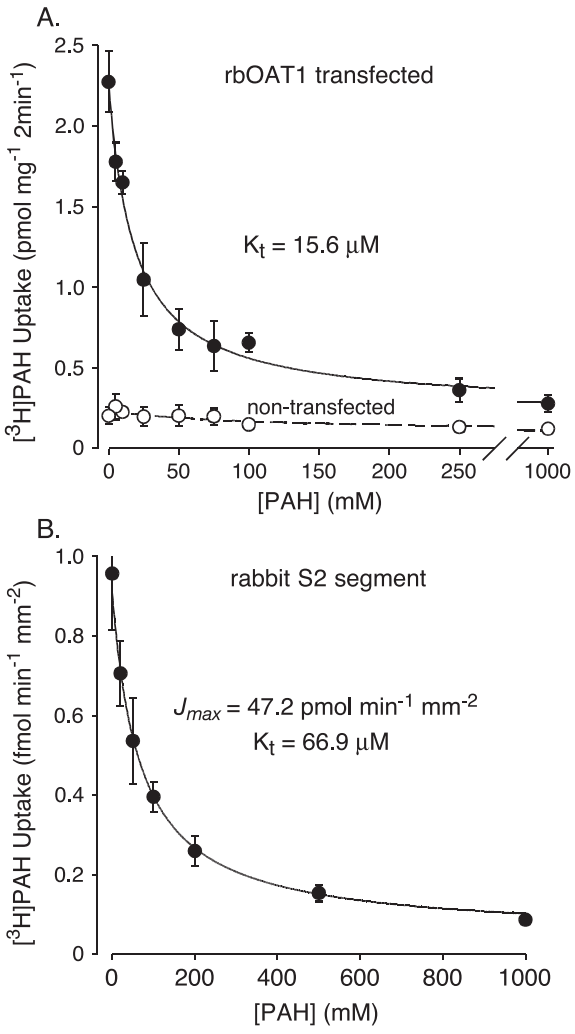

Fig. 2. (A) Kinetics of PAH transport by rbOAT1 expressed in COS7 cells. Uptake of radiolabeled PAH ([^{3}H]PAH) (0.2 μM) on ordinate vs. concentration of unlabeled PAH in bathing medium on abscissa. Values are means ± S.E. n = 3 experiments. Solid circles represent transport in transfected cells; open circles represent transport in non-transfected cells. Figure is redrawn with modifications from Ref. [26]. (B) Kinetics of PAH transport by S2 segments of rabbit proximal renal tubules. Uptake of radiolabeled PAH ([^{3}H]PAH) (6 μM) on ordinate vs. concentration of unlabeled PAH in bathing medium on abscissa. Values are means ± S.E. n = 3 tubules. Symbols are the same as in A. Preliminary unpublished data from A. Lungkaphin.

the same as the response observed previously when intact, isolated rabbit S2 segments were exposed to αKG in the bathing medium (Fig. 3) [15]. The uptake of αKG by the Na-DC cotransporter is sufficiently rapid in the intact tubule that the stimulation of the initial rate of FL uptake via the OA/αKG exchanger occurs within about 5 s of exposure to physiological levels of αKG (10 μM; Fig. 4) [15]. As shown in Fig. 3, we also determined the effect of preloading the rbOAT1-transfected COS7 cells with urate on the initial rate of FL uptake. Our reason for doing this was that rabbit proximal tubules show net secretion of urate and there was some evidence that such secretion might involve the "classical" OA secretory pathway [34]. If so, it should show countertransport for FL. However, as shown in Fig. 3, preloading the COS7 cells with urate had no effect on FL uptake. Thus, the cloned rbOAT1 transporter, at least as expressed in a heterologous system, does not appear to support a substantial rate of urate transport.

3.2. OAT3

In examining the cloned rbOAT3, we not only considered possible transport of PAH but also the transport of estrone sulfate (ES), which has been shown to interact with cloned OAT3 from other species (e.g., [35]). Initially, we simply examined the degree of uptake of these substrates by rbOAT3-transfected CHO cells compared to wild-type CHO cells. As shown in Fig. 5, rbOAT3 expressed in CHO cells, unlike rbOAT1 expressed in COS7 cells, had almost no ability to transport PAH. However, it readily transported radiolabeled ES and this transport was completely inhibited by a high concentration of unlabeled ES (Fig. 5). Preliminary data now indicate that rbOAT1 expressed in COS7 does support modest ES transport, but the affinity of rbOAT1 for ES is comparatively quite low. Thus, these two rabbit transporters have strikingly different substrate selectivities, at least when expressed in heterologous systems. In addition, rbOAT3 expressed in CHO cells readily transported cimetidine (Fig. 5). This is interesting because cimetidine appears capable of being transported by both the organic anion and the organic cation transport systems (e.g., Ref. [36]), and this observation suggests that renal secretion by the "classical" organic cation pathway may involve interaction with OAT3, in rabbits at least.

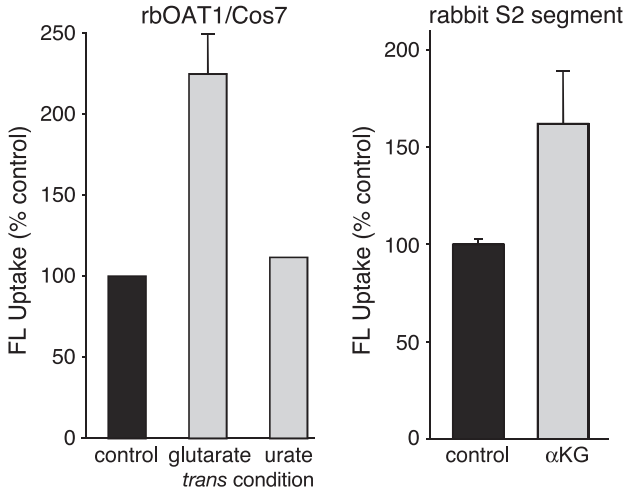

Fig. 3. Left-hand panel. FL (1 μM) uptake as percentage of control (with control being 100%) by rbOAT1 expressed in COS7 cells before (control) and after preloading (*trans* condition) with glutarate or urate. Bars represent means ± S.E. n = 3 experiments. Figure is redrawn with modifications from Ref. [16]. Right-hand panel. FL (1 μM) uptake as a percentage of control (with control being 100%) in the absence (control) and presence of 10 μM αKG. Symbols are the same as in the left-hand panel. n = 18 tubules. Redrawn with modifications from Ref. [15].

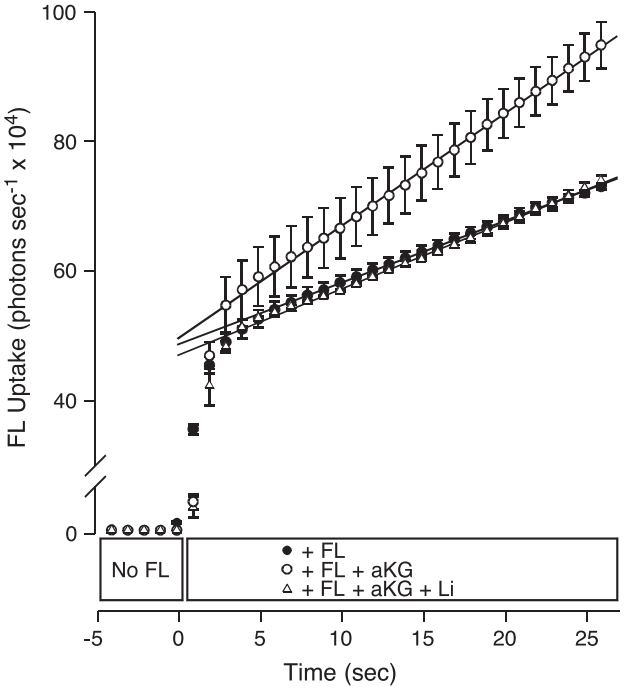

Fig. 4. Fluorescence record showing stimulation of FL uptake into an S2 segment of rabbit proximal renal tubule resulting from an acute *cis*-exposure to 1 μM FL plus 10 μM αKG and blockage of that *cis*-stimulation by simultaneous exposure to 2 mM Li^+ (which blocks αKG uptake by the Na^+-DC cotransporter). FL uptake is expressed in terms of photomultiplier output. Fluorescence output prior to $t=0$ (period labeled "No FL") reflects autofluorescence of tubule. At time 0, superfusion buffer was switched to one containing 1 μM FL. In all measurements, there was a rapid increase in fluorescence (to ~ 450,000 photons/s) due to the addition of FL and a subsequent slower increase in fluorescence from the accumulation of FL in the tubule. Each point is means ± S.E of three determinations of FL uptake (where error bars are not evident, the error was smaller than the graphic representation of the mean). Solid lines are linear regressions determined from increases in fluorescence between $t=5$ s and $t=25$ s. From Ref. [15] with permission.

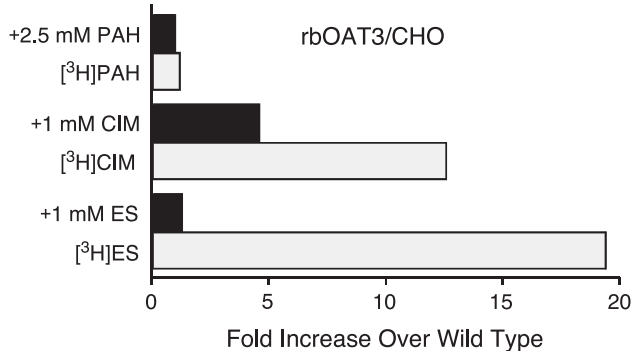

Fig. 5. Uptake of radiolabeled PAH ([^{3}H]PAH) (0.25 μM), cimetidine ([^{3}H]CIM) (0.10 μM), and estrone sulfate ([^{3}H]ES) (0.023 μM) and effects of high concentrations of unlabeled substrates on it by rbOAT3 expressed in CHO cells. Data shown are multiples of uptake by wild-type CHO cells. Bars represent mean of three experiments. Preliminary unpublished data from X. Zhang.

Because ES appeared to be such an excellent substrate for rbOAT3, we examined the kinetics of its transport, first by determining the uptake of radiolabeled ES by CHO cells expressing rbOAT3 with increasing concentrations of unlabeled ES and second by making similar determinations with isolated rabbit S2 proximal tubule segments with increasing concentrations of unlabeled ES. As shown in Fig. 6, ES transport saturated with a K_t of ~ 4 μM that, unlike the K_t for PAH transport by rbOAT1, was the same for ES transport by rbOAT3 expressed in CHO cells and in intact, rabbit proximal S2 tubules.

For rbOAT3, it was particularly important to determine that the transport we were observing involved OA/αKG countertransport because only recently has OAT3 from any

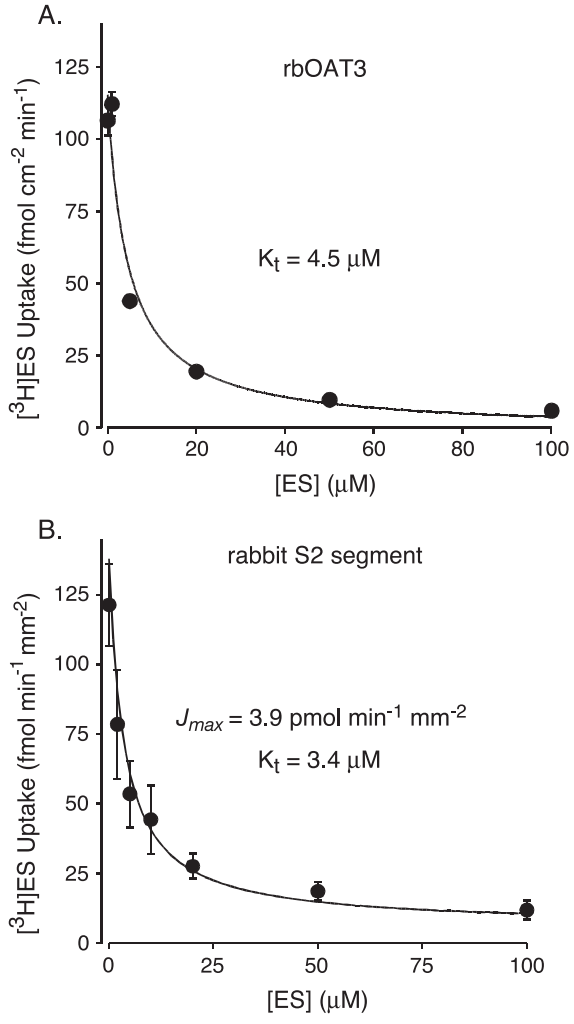

Fig. 6. (A) Kinetics of ES transport by rbOAT3 expressed in CHO cells. Uptake of radiolabeled ES ([^{3}H]ES) (0.023 μM) on ordinate vs. concentration of unlabeled ES in bathing medium on abscissa. Values are means from three wells from a single experiment. Unpublished data from X. Zhang. (B) Kinetics of ES transport by S2 segments of rabbit proximal renal tubules. Uptake of radiolabeled ES ([^{3}H]ES) (1 μM) on ordinate vs. concentration of unlabeled ES in bathing medium on abscissa. Values are means ± S.E. $n=3$ tubules. Preliminary unpublished data from A. Lungkaphin.

species been shown to perform such countertransport [3]. For this purpose, we measured the initial rate of uptake of radiolabeled ES by CHO cells expressing rbOAT3 and isolated rabbit proximal S2 segments under control circumstances and when each preparation was preloaded with glutarate (as a non-metabolized surrogate for αKG). As shown in Fig. 7, preloading with glutarate led to a doubling of the rate of ES uptake in both the CHO cells and proximal S2 segments, thereby demonstrating that rbOAT3 can function as an appropriate countertransporter for the final step in the "classical" tertiary active OA transport pathway across the basolateral membrane (Fig. 1).

Of additional interest, preloading CHO cells expressing rbOAT3 with urate stimulated uptake of radiolabeled ES even more effectively than preloading the cells with glutarate (Fig. 7). These data indicate that urate can be transported by rbOAT3 and this appears to be confirmed by recent preliminary studies on direct transport of radiolabeled urate by rbOAT3 expressed in CHO cells.

3.3. Summary comparison of function of rbOAT1 and rbOAT3

Based on our studies to date, both rbOAT1 and rbOAT3 can function as OA/αKG countertransporters in the "classical" tertiary active pathway for transport of OAs across the basolateral membrane of the renal tubules (Fig. 1). However, the substrate specificity of these two transporters appears to be strikingly different. Whereas rbOAT1 readily transports PAH and barely transports ES, rbOAT3 avidly transports ES and effectively has no interaction with PAH. This appears to be the case not only when these transporters are artificially expressed in heterologous systems, but also when they are naturally expressed in intact, isolated S2 segments of rabbit proximal tubules. Indeed, we have compared the inhibition of radiolabeled PAH transport by unlabeled ES and radiolabeled ES transport by unlabeled PAH in these isolated tubule segments. In these studies, the ratio of the IC_{50} for ES inhibition of PAH transport to the K_t for ES transport was ~ 25 and the ratio of the IC_{50} for PAH inhibition of ES transport to the K_t for PAH transport was ~12. If the transport pathways for these two substrates involved any significant interaction, these ratios should have been close to 1.0. Therefore, it appears that in the S2 segment of the rabbit proximal tubule PAH is transported by a process (presumably OAT1) different from that for ES (presumably OAT3).

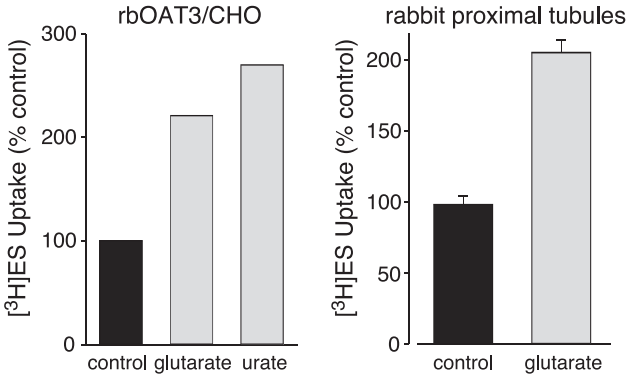

Fig. 7. Left-hand panel. Radiolabeled ES ([^{3}H]ES) (0.023 μM) uptake as a percentage of control (with control being 100%) by rbOAT3 expressed in CHO cells before (control) and after preloading (*trans* condition) for 15 min with glutarate (500 μM) or urate (1 mM). Bars represent mean of three wells from a single experiment. Preliminary unpublished data from X. Zhang. Right-hand panel. Radiolabeled ES ([^{3}H]ES) (1 μM) in suspension of rabbit proximal renal tubules as a percentage of control (with control being 100%) before (control) and after preloading for 15 min with 500 μM glutarate. Bars represent means ± S.E. of duplicate of a single experiment. Preliminary unpublished data from C. Groves.

Moreover, the affinity of rbOAT3 for ES is substantially greater than that of rbOAT1 for PAH (Figs. 2 and 6). And the difference between the K_ts for PAH interaction with rbOAT1 in COS7 cells and in proximal S2 segments (Fig. 2) cannot be explained by interaction with rbOAT3 in the tubule segments. It is also of interest that our current preliminary data suggest that secretion of urate and cimetidine by S2 segments of rabbit proximal tubules may involve primarily OAT3, not OAT1. However, it is also becoming apparent that these two transporters do not necessarily have different affinities for all substrates. For example, preliminary data with the transporters in heterologous expression systems and in S2 segments of rabbit proximal tubules suggest that both transporters have about the same affinity for FL.

The combination of the differences and similarities in substrate affinities now make it possible to functionally map the transporters along the proximal tubule. At present, we have only begun this process with the S2 segment of the rabbit proximal tubule. This is the segment that, on the basis of PAH transport, has been considered to be the primary site of OA secretion in the mammalian renal tubules [6–8]. Indeed, it clearly does transport PAH via OAT1. Moreover, the observation that the J_{max} for basolateral uptake of radiolabeled PAH by rabbit S2 proximal segments is about 12 times the J_{max} for basolateral uptake of radiolabeled ES by these same segments (Figs. 2 and 6) suggests that there are more OAT1 transporters than OAT3 transporters in this segment. However, this requires further study, as do the transport properties of the S1 and S3 segments. In any case, our current data suggest that the transport of a given substrate in any tubule segment depends on both the affinity of each transporter that can accept that substrate as well as the level of expression of each of those processes in that particular tubule segment.

4. Regulation of basolateral organic anion transport

Although regulation of the basolateral exchanger can be examined with cloned transporters expressed in heterologous systems, it is not possible to know whether apparent regulation under these circumstances reflects the situation

when the transporter is expressed in its native membrane environment within an intact epithelium. Therefore, we are in the process of determining regulation within intact, isolated proximal tubule segments. However, only within the past year has it been recognized that OAT3 as well as OAT1 can function as the basolateral exchanger within the intact renal tubule and that the substrate specificities of these two transporters differ (see above) [3,35]. Therefore, much of our work (and that of others) did not take the possibility of two transporters into account. Indeed, by the middle 1990s, even before OAT1 was cloned and found to have consensus sites for phosphorylation by PKC (thereby making it a potential target for regulation via pathways involving activation of PKC; see above), a few studies with both mammalian and non-mammalian cells and tubules suggested that phosphorylation via PKC could be important [37–39]. Although most data suggested that activation of PKC depressed OA transport, there were some discrepancies in the data. We re-evaluated and extended these observations by examining both the initial rate of uptake and transepithelial transport of FL in real time with isolated non-perfused and perfused S2 segments of rabbit proximal tubules [40,41]. Our data indicate that activation of PKC, either directly with phorbol 12-myristate 13-acetate (PMA) or 1,2-dioctanoyl-*sn*-glycerol (DOG) or indirectly with ligands of physiological receptors coupled to the PKC pathway (α_1-receptor agonist phenylephrine or peptide hormone bradykinin), inhibits basolateral FL uptake and transepithelial transport. This effect is prevented by the PKC-specific inhibitor bisindolylmaleimide I (BIM). Thus, in intact S2 segments of rabbit renal tubules, physiological stimuli can reduce basolateral OA uptake via the PKC pathway. However, since these studies utilized FL as the transported substrate, we now know that this down-regulation could have involved OAT1, OAT3, or both. Because PKC activation has been shown to down-regulate OA transport by several orthologs of OAT1 expressed in heterologous systems (hOAT1 expressed in HeLa cells [21]; rOAT1 expressed in oocytes [42]; mOAT1 expressed in LLC-PK1 cells [43]) and by rOAT3 expressed in oocytes [44], we suspect that down-regulation by PKC-activation in these intact rabbit S2 proximal segments involves both OAT1 and OAT3. Nevertheless, this needs to be demonstrated by studies with intact epithelia involving substrates specific for each transporter (i.e., PAH and ES).

We, with our collaborators in Würzburg, Germany, Michael Gekle and Christoph Sauvant, have continued to undertake a more detailed evaluation of possible regulation of basolateral PAH transport by more physiological activation of regulatory pathways. We have been concentrating on epidermal growth factor (EGF), which has been suggested as a mediator of normal tubular growth and tubular regeneration after injury. EGF and its receptor are expressed in rabbit proximal tubule cells [45]. However, the initial studies involved the effects of EGF on PAH transport by opossum kidney (OK) cells in culture, a proximal tubule-derived cell line known to demonstrate OA transport and to contain the mRNA for OAT1 which is simpler to work with than isolated, individual renal tubules [46]. In this preparation, Sauvant et al. [46] showed that EGF stimulates basolateral PAH uptake through the mitogen-activated protein kinase (MAPK) pathway via sequential activation of mitogen-activated/extracellular-signal regulated kinase kinase (MEK) and extracellular signal-regulated kinase isoforms 1 and 2 (ERK1/2). The stimulatory effect of EGF on PAH uptake is completely blocked by inhibitors of MEK (PD98059 or U0126). Western blots of OK cell protein showed phosphorylation of ERK1/2 with EGF administration that was eliminated by inhibition of MEK, results that exactly paralleled the effects on PAH transport. Our current follow-up studies with individual isolated rabbit S2 proximal tubule segments show an identical pattern for both inhibition of PAH uptake by U0126 (Fig. 8) and Western blots of ERK1/2 phosphorylation, except for indicating that the MAPK pathway apparently also plays a role in the baseline levels of PAH uptake. Since these studies involved PAH transport, we know that these steps in up-regulation via EGF in the intact rabbit S2 segment involve OAT1, but we do not know if they apply to OAT3. We also do not know if they apply to the S1 and S3 proximal tubule segments.

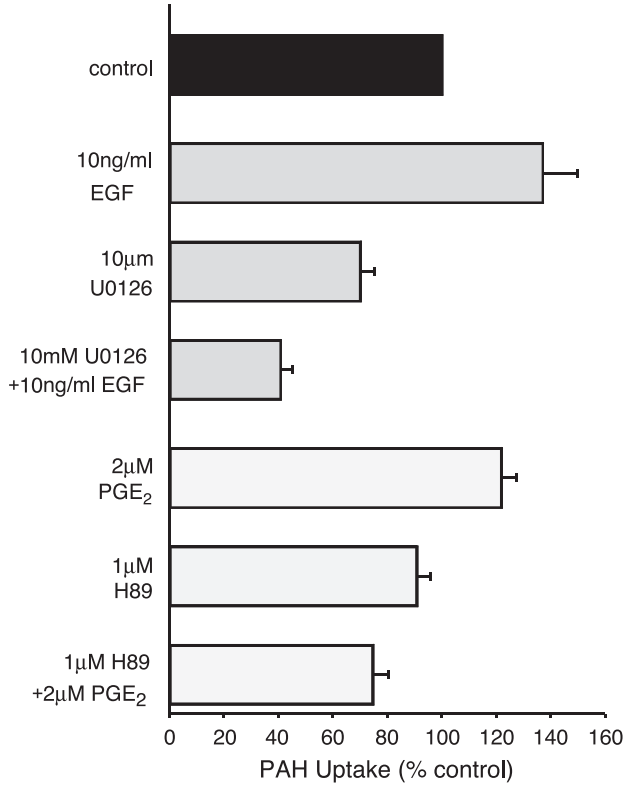

Fig. 8. Radiolabeled PAH ([^{3}H]PAH) (6 μM) uptake as a percentage of control (with control being 100%) by S2 segments of rabbit proximal tubules. Bars represent means ± S.E. for 12 tubules for EGF and U0126 data and 8 tubules for PGE_2 and H89 data. Treatments are shown below bars. Preliminary unpublished data from W.H. Dantzler.

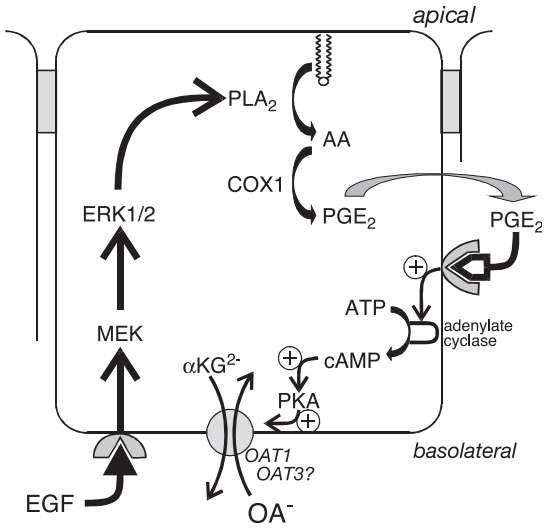

Fig. 9. Model of apparent regulatory cascade for stimulation of organic anion transport by EGF. Abbreviations: mitogen-activated/extracellular-signal regulated kinase kinase (MEK); extracellular-signal regulated kinase isoforms 1 and 2 (ERK1/2); phospholipase A_2 (PLA_2); arachidonic acid (AA); cyclooxygenase 1 (COX1); prostaglandin E_2 (PGE_2); protein kinase A (PKA). Other symbols are the same as in Fig. 1. Question mark indicates uncertainty about application to OAT3.

We are extending these studies to investigate the regulatory network of EGF action on PAH uptake downstream of ERK1/2 in more detail. The initial studies again involved OK cells in culture [47]. Using a series of inhibitors and an assay for arachidonic acid release, Sauvant et al. concluded that EGF successively activates MEK, ERK1/2, and phospholipase A_2 (PLA_2), leading to an increased release of arachidonic acid. Subsequently, arachidonic acid is metabolized to prostaglandins via cyclooxygenase 1 (COX1), which then mediate EGF-induced stimulation of basolateral PAH uptake. Additional preliminary studies indicate that prostaglandin E_2 (PGE_2) stimulates basolateral PAH uptake in OK cells via adenylate cyclase activation and subsequent PKA activation (C. Sauvant and M. Gekle, personal communication). We are continuing these studies with isolated rabbit S2 proximal tubule segments, in which we find that PGE_2 also stimulates basolateral PAH uptake and that this stimulation is prevented by the PKA inhibitor H89 (Fig. 8). Thus, this cascade apparently functions in intact S2 segments of rabbit proximal tubules (see summary in Fig. 9). As discussed above, transport of PAH by rabbit tubules probably reflects activity of OAT1. There is currently no evidence concerning the influence of activation of the MAPK signaling cascade on activity of OAT3. Moreover, we have no idea whether this regulatory cascade applies to the S1 and S2 proximal segments. Studies with transporter-specific substrates (e.g., PAH and ES for OAT1 and OAT3, respectively) and all three tubule segments are needed to resolve this situation. Knowledge of the extent to which regulation of OAT1 and OAT3 activity influences the profile of renal organic anion secretion in intact renal proximal tubules is critical to understanding the regulation of the basolateral transport of both toxic and therapeutic xenobiotics.

Finally, it must be noted that despite the presence of PKC and PKA phosphorylation sites on the OAT1 and OAT3 transporters, it is not certain that either down-regulation through PKC activation or up-regulation through PKA activation actually involves phosphorylation of the transporters. Indeed, in the only study to date to examine this issue, activation of PKC in LLC-PK1 cells that stably expressed mouse OAT1 did not result in phosphorylation of OAT1 protein. The decrease in PAH uptake that followed activation of PKC in these cells was associated with a decrease in J_{max}, with no change in K_t. These observations led to the suggestion that activation of PKC may increase internalization of membrane transporters, or inhibit recruitment of preformed transporters from a cytoplasmic pool [43]. Similar kinetics (reduction of J_{max} with no change in K_t) are seen for the inhibition of the activity of heterologously expressed rOAT3 by PKC activation [44], and this may be the case in the intact renal tubules.

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